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Pharmacokinetics of salsalate and salicylic acid in normal and diabetic rats

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Abstract

The pharmacokinetics (PK) of salsalate (SS) and salicylic acid (SA) was assessed in normal Wistar and diabetic Goto-Kakizaki rats. Three PK studies were conducted: 1) PK of SA in normal rats after intravenous dosing of SA at 20, 40, 80 mg/kg. 2) PK of SS and SA in normal rats after oral dosing of SS at 28, 56, 112 mg/kg. 3) PK during 4 months feeding of SS-containing diet in both normal and diabetic rats. The disposition of SS and SA were simultaneously evaluated using a pharmacokinetic model comprised of several transit absorption steps and linear and nonlinear dual elimination pathways for SA. The results indicated that the nonlinear elimination pathway of SA only accounted for a small fraction of the total clearance (< 12%) at therapeutic concentrations. A flat profile of SA was observed after oral dosing SS, particularly at a high dose. The possible reasons for this flat profile were posed. During the SS-diet feeding, diabetic rats achieved lower blood concentrations of SA than normal rats with a higher apparent clearance (CL/F) possibly due to incomplete (47%) bioavailability. Such CL/F decreased with age in both diabetic and normal rats. The effect of diabetes on SA pharmacokinetics may necessitate increased dosing in future usage of SS in diabetes.

Keywords

salsalate; salicylic acid; pharmacokinetics; diabetes

Introduction

Salsalate (SS), a dimer of salicylic acid (SA), has been widely used as an analgesic and anti-inflammatory agent for about 100 years. Recently, several clinical trials indicated that SS has favorable effects in type 2 diabetes (T2D) [1-3], probably via inhibition of the chronic inflammation that widely occurs [4, 5]. These beneficial effects of SS in type 2 diabetes include the reduction of hyperglycemia, triglycerides, free fatty acids, as well as C-reactive proteins [6]. The potential usage of SS in T2D has attracted public attention, as the drug is well tolerated and considered safe after decades of clinical use. The potential use of SS in diabetes therapy must consider whether the pharmacokinetics of SS and SA are altered. This study compares SS and salicylate pharmacokinetics in normal Wistar and diabetic Goto-Kakizaki rats (GK, T2D rats).

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Previously, the pharmacokinetics (PK) of SA was investigated in both rats and humans and nonlinear clearance was reported [7]. The underlying mechanism of the nonlinear clearance was primarily the limited capacity for synthesis of salicylurate from salicylate and glycine. The salicylurate formed was excreted into urine, which made it possible to directly assess this nonlinear component by measuring its appearance rate. Nelson et al determined the maximum capacity of this nonlinearity to be 300 ug/kg in rats [7]. We used this estimate to improve model stability. Previous studies indicated that the PK of SA was influenced by physiological factors such as age, sex, pregnancy, renal function, and protein-calorie malnutrition [8]. Factors such as altered renal function and protein status are frequently intertwined with T2D. Thus, SA might show different pharmacokinetic behavior in diabetic animals.

Although SS is widely used clinically, its oral absorption behavior and in vivo hydrolysis process have been only sparingly reported. One study indicated that the hydrolysis of SS to SA was more efficient than aspirin in vivo [9]. However, the hydrolysis rate has never been clearly established. This report describes the PK of SS and SA in normal and diabetic rats. Our studies were designed to support preclinical use of SS to treat T2D in GK rats, a common animal model for this disease [4].

Materials and Methods

Animals

Twenty-two 12-week-old male Wistar rats (334 ± 28 g) were obtained from Harlan (Indianapolis, IN) for single dose studies. Six 4-week-old male GK rats (65 ± 9 g) were purchased from Taconic Farms (Germantown, NY) and six 4-week-old male Wistar rats (64 ± 6 g) were obtained from Harlan for the feeding study. GK rats showed higher body weights than age-matched Wistar rats throughout the diet feeding study [4]. All rats were maintained on a strict 12-h light/12-h dark cycle, had free access to food and water, and were acclimatized for 1 week before studies.

All animal studies were approved by the Institutional Animal Care and Use Committee of the University at Buffalo.

Diet

The drug was formulated in AIN-6A diet with bacon flavor (TestDiet, Richmond, IN), which included 8.0% protein, 5.0% fat, and 65.4% carbohydrate and had a digestible energy content of 3.77 kcal/g, with approximately 70% energy from carbohydrates. The concentration of SS in the diet was 1000 ppm.

Experiments

Study 1. Pharmacokinetic study of SA in normal rats.

All rats underwent surgery to externally cannulate the jugular vein one day before the PK study. The 11 rats were randomly divided three groups for SA doses of 20, 40, and 80 mg/kg. Blood was collected before and at 0.5, 1, 2, 4, 6, 8, 12, 24 hr after intravenous (IV) dosing.

Study 2. Pharmacokinetic study of SS in normal rats

After jugular vein cannulation, the rats were orally gavaged with SS (suspension in 30% PEG 400) at doses of 28, 56, and 112 mg/kg. Blood was obtained before and at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 16, 24, 33, 48 hr after dosing. These points were evenly

assigned to these rats to assure each time point included at least three rats and that no rat would be oversampled.

Study 3. Pharmacokinetic study by feeding SS-diet in normal and diabetic rats.

The 6 GK rats and 6 Wistar rats were fed with the SS-containing diet from 5 to 19 weeks of age. The food was changed every other day and leftover material was measured to assess food intake. Body weight was measured every other day. The food intake X SS concentration was used to calculate SS dosages. Blood (45 ul) was collected once a week for drug analysis.

Analysis method

Blood salicylates were measured using a published high-performance liquid chromatography method with minor modifications (lowering the mobile flow from 2.0 to 1.5 ml/min for system protection) [10]. The quantification limits were 1.6 ug/ml for both SS and SA with less than 10% intra-batch variability.

Models

Fig. 1 shows the final structure of the PK model. A series of transit steps ($n = 4$) was employed to describe the gradual absorption process [11]:

$$dABS_{1/dt} = -(k_r + k_a) \cdot ABS_1 \quad ABS_1(0) = 0 \quad (1)$$

$$dABS_{N/dt} = k_r \cdot ABS_{N-1} - (k_r + k_a) \cdot ABS_N \quad ABS_N(0) = 0 \quad (2)$$

where ABS_N is the SS amount in the n th transit step, k_r is the transit rate constant, and k_a is the absorption rate constant.

The kinetics of SS was described by:

$$dA_{cs}/dt = Input_{ss} - (CL_{ds}/V_{cs} + k_{met}) \cdot A_{cs} + CL_{ds}/V_{ps} \cdot A_{ps} \quad A_{cs}(0) = 0 \quad (3)$$

$$dA_{ps}/dt = CL_{ds}/V_{cs} \cdot A_{cs} - CL_{ds}/V_{ps} \cdot A_{ps} \quad (4)$$

$$Input_{ss} = (ABS_1 + ABS_2 + ABS_3 + ABS_4) \cdot k_a \cdot f_r \quad (5)$$

where A_{cs} and A_{ps} are SS amounts in central (V_{cs}) and peripheral (V_{ps}) compartments, CL_{ds} is the distribution clearance, k_{met} is the hydrolysis rate of SS into SA, $Input_{ss}$ is the collective absorption rate from the four transit steps, and f_r is the fraction that is absorbed without hydrolysis.

Linear and nonlinear mixed clearance was applied to describe SA PK as:

$$dA_{ca}/dt = Input_{sa} + \varphi \cdot A_{cs} \cdot k_{met} + \frac{CL_{da}}{V_{pa}} \cdot A_{pa} - \left(\frac{CL_{da}}{V_{ca}} + \frac{V_m \cdot A_{ca}/V_{ca}}{K_m + A_{ca}/V_{ca}} + CL_a/V_{ca} \right) \cdot A_{ca} \quad A_{ca}(0) = 0 \quad (6)$$

$$dA_{pa}/dt = CL_{da}/V_{ca} \cdot A_{ca} - CL_{da}/V_{pa} \cdot A_{pa} \quad A_{pa}(0) = 0 \quad (7)$$

$$Input_{sa} = (ABS_1 + ABS_2 + ABS_3 + ABS_4) \cdot k_a \cdot (1 - fr) \quad (8)$$

where A_{ca} and A_{pa} are the amounts of SA in central (V_{ca}) and peripheral (V_{pa}) compartments, CL_{da} is the distribution clearance, ϕ is a correction factor for the molecular weight difference between SS and SA (1.07), $Input_{as}$ is the collective absorption rate from the four transit sites, and $1-f_r$ is the fraction that is hydrolyzed during absorption. The $Input_{sa}$ and $A_{cs} \cdot k_{met}$ were not applied to the IV dosing study. The V_m is the capacity of the nonlinear elimination pathway of SA and K_m is the SA concentration at 50% of V_m .

Alternative models tested include differing numbers of compartments, transit steps, and type of elimination processes.

The change of apparent clearance with age was described as:

$$CL/F = CL_0/F - (CL_m/F) \cdot T / (TA_{50} + T) \quad (9)$$

where CL_0/F is initial value of apparent clearance at 5 weeks age, T is age (or time), CL_m/F represents the extent of maximum decline of apparent clearance with age, and TA_{50} is the age when 50% maximum decline occurs.

Data Analysis

The ADAPT 5 program with the maximum likelihood method was used for all modeling [12]. Naive-pooled data from all animals was used to fit the model and a linear variance model was used in all fittings as:

$$V_i = (\sigma_1 + \sigma_2 Y(t_i))^2 \quad (10)$$

where V_i is the variance of the response at the i th time point (t_i) and $Y(t_i)$ represents the predicted response at time t_i from the model. Variance parameters σ_1 and σ_2 were estimated together with system parameters during fittings. The goodness-of-fit criteria included visual inspection of the fitted curves, sum of squared residuals, Akaike information criterion (AIC), Schwartz Criterion (SC), and Coefficients of Variation (CV) of the estimated parameters.

Results and Discussion

The PK profiles of SA after IV dosing are shown in Fig. 2. The fitted parameters are listed in Table 1. The profiles of SA showed biexponential disposition. The volume of the central compartment (V_{ca}) always converged close to plasma volume in the model fittings; thus V_{ca} was fixed to plasma volume in the final model to improve model stability [13]. This small volume indicated slow distribution of SA, which is in line with a small CL_{da} (158 ml/hr/kg) compared with rat cardiac output (17760 ml/hr/kg) [13]. The slow distribution is probably associated with high protein binding of SA in blood (about 90% at 100 ug/mL) [14].

Classical nonlinear elimination is seen in Fig. 2, with lower concentrations showing more rapid decline. Such nonlinear clearance of SA has been reported previously [7]. Although the nonlinearity is clearly observed in Fig. 2, the relative fraction of this nonlinear clearance to total clearance is low according to our estimation, no more than 12% at therapeutic concentrations (> 100 ug/mL). The small degree of nonlinearity indicated the limited influence of glycine in the disposition of SA, which is consistent with previous observations [7]. The present data weakly supported the simultaneous estimation of all these parameters; V_m had to be fixed to a literature value derived from urinary excretion of salicylurate [7].

The PK profiles of SA and SS after orally dosing SS are displayed in Fig. 3. Table 1 summarizes the parameter estimations. For SS PK, besides hydrolysis into SA, another elimination pathway was tried in the original model but the fitting always gave a small value of this component. Therefore, hydrolysis was solely considered in the final model for SS elimination. Compared with SS, SA exhibited a flat profile, particularly at the high dose. Deconvolution analysis suggested a prolonged input. The reason for this input is not clear. The model fitting results suggested that a slow absorption process and a sustained hydrolysis from SS are most likely causes.

As shown in Fig. 1, a series of transit steps was used to describe the gradual absorption process. The optimal transit number was 4, and the transit time was estimated as 1.46 hr ($1/k_p$). The total residence time of the four transit steps ($4/k_p$) was estimated to be 5.8 hr, in the range of intestinal transit time [15]. Although SS was reported to be readily absorbed from the intestine, the sluggish absorption process in this study was probably caused by the PEG suspension. The viscous PEG suspension may have either impeded the dispersion of SS in the intestine and slowed absorption [16] or reduced the membrane permeability of the drug [17].

The prolonged input of SA may also be related to the hydrolysis of SS. Such hydrolysis occurs via esterases in the gastrointestinal tract, liver, blood and other tissues. Abundant esterases in the blood produce rapid hydrolysis of SS to SA. This is reflected by a high value of k_{met} in our analysis. However, sustained redistribution of SS from the peripheral tissues also occurs. The value of V_{ps} is large and SS would return relatively slowly ($CL_{ds}/V_{ps} = 0.066 \text{ hr}^{-1}$) to blood. This appears to be the rate-limiting step in controlling the hydrolysis process of SS. A PK study with IV dosing of SS would be required to confirm this.

In our diet dosing study, no SS was detected in any blood samples. The SA blood concentrations and food consumption are shown in Fig. 4. The SA blood concentrations decreased with increasing age, from 156 to 98 ug/mL for Wistar rats over 5 to 19 weeks of age, and from 129 to 87 ug/mL for GK rats over this period. The GK rats had lower blood concentrations of SA over all ages. The effective concentrations in treatment of T2D have not been well established. Our previous study observed a significant effect of SA in reducing diabetes disease progression at SA concentrations of 72-130 ug/ml (these data), but no concentration-effect relationship was assessed [4]. Food consumption also declined over time, from 261 to 93 g/kg/day for Wistar rats and from 298 to 89 g/kg/day for GK rats. The absolute bioavailability (F) of SA from food could not be accurately determined, but an estimate could be made according to $(AUC_{diet}/Dose_{diet})/(AUC_{iv}/Dose_{iv})$ using the measured concentrations as steady-state averages. The F was estimated at 47.4% for the Wistar rats at 12 weeks of age. Assuming F was consistent over time and across species, the apparent clearance of SA was estimated according to $CL/F = Dose/C_{sa} = Food \cdot 1000 \text{ ppm}/C_{sa}$. The changes of apparent clearance over time are shown in Fig. 5, with quantitative description by equation (9). Young Wistar and GK rats both had higher clearances of SA compared with aged rats. This is consistent with observations in Sprague-Dawley rats [8]. Interestingly, diabetic rats exhibited higher apparent clearances of SA than normal rats. The apparent clearances approach comparable levels by the age of 19 weeks. This suggests that diabetic status enhances SA clearance. If this also applies in humans, a higher dose than used in non-diabetic subjects might be required to achieve the same therapeutic concentrations. T2D is accompanied by decreased protein binding of many drugs and reduced renal function [18]. These factors may affect SA PK in opposing ways. Quantitatively assessing the influence of these factors would be important for using SS in treatment of diabetes in patients.

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Fig. 1.
Pharmacokinetic model for salicylic acid and salsalate. Symbols are defined in the text and Table 1.

Fig. 2.
Pharmacokinetic profiles of salicylic acid after intravenous doses in normal rats.

Fig. 3.
Pharmacokinetic profiles of salsalate and salicylic acid after oral dosing SS at three doses in normal rats.

Fig. 4. Blood salicylic acid concentrations and food intake during 15 weeks diet feeding in normal Wistar and diabetic GK rats.

Fig. 5. Changes of apparent clearance of salicylic acid with age in normal Wistar and diabetic GK rats.

Table 1

Pharmacokinetic parameters of salsalate (SS) and salicylic acid (SA).

Parameter	Unit	Definition	Values	CV(%)
<u>Salicylic Acid</u>				
V_{ca}	mL/kg	Central distribution volume	31.2	NA ^a
V_{pa}	mL/kg	Peripheral distribution volume	192	4.35
CL_a	mL/hr/kg	Linear clearance	20.6	3.66
V_m	ug/hr/kg	Maximum elimination rate	300	NA ^a
K_m	ug/mL	Concentrations at 50% of V_m	0.697	44.1
CL_{da}	mL/hr/kg	Distribution clearance	158	4.95
<u>Salsalate</u>				
k_a	1/hr	Absorption rate	0.236	3.28
CL_{ds}	mL/hr/kg	Distribution clearance	289	7.93
V_{cs}	mL/kg	Central distribution volume	31.2	NA ^a
V_{ps}	mL/kg	Peripheral distribution volume	4349	18.0
k_{met}	1/hr	Biotransformation rate of SS to SA	15.5	31.3
k_y	1/hr	Transfer rate between transit compartments	0.686	5.34
f_r	/	First-pass transformation fraction	0.969	13.7

Note:

^aNot Applicable.

Table 2
Parameters describing decreased apparent clearance of salicylic acid (SA) with age

Parameter	Definition	GK rat		Wistar rat	
		Mean	CV%	Mean	CV%
CL_o/F (mL/hr/kg)	Apparent clearance at 5 weeks age	94.6	4.10	68.0	6.57
CL_m/F (mL/hr/kg)	Maximum decrease magnitude of apparent clearance of SA	53.2	8.88	32.3	20.0
CL_{last}/F (mL/hr/kg)	The minimum apparent clearance of SA with age	41.4	14.2 ^a	35.7	18.0 ^a
TA_{50} (week)	The age when half maximum decline of apparent clearance was observed	7.24	33.2	10.38	73.5

Note:

^a secondary parameter of CL_o/F - CL_m/F .