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Hepatic structure-pharmacokinetic relationships: The hepatic disposition and metabolite kinetics of a homologous series of O-acyl derivatives of salicylic acid

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1 The hepatic disposition and metabolite kinetics of a homologous series of O-acyl (acetyl, propionyl, butanoyl, pentanoyl, hexanoyl and octanoyl) esters of salicylic acid (C2SA, C3SA, C4SA, C5SA, C6SA and C8SA, respectively) was determined using a single-pass, *in-situ* rat liver preparation.

2 The hepatic venous outflow profiles for the parent esters and the generated metabolite, salicylic acid (SA) were analysed by HPLC. Non-parametric moments analysis was used to determine the area under the curve (AUC′), mean transit time (MTT) and normalized variance (CV^2) for the parent esters and generated SA.

3 Pregenerated SA ([^{14}C]-salicylic acid) was injected into each liver with the parent ester to determine its distribution characteristics.

4 The overall recovery of ester plus metabolite was 89% of the ester dose injected and independent of the ester carbon number, suggesting that ester extraction was due to hepatic metabolism to salicylic acid.

5 The metabolite AUC′ value increased directly with the lipophilicity of the parent ester (from 0.12 for C2SA to 0.95 for C8SA). By contrast, the parent AUC′ decreased with the lipophilicity (from 0.85 for C2SA to zero for C8SA). The metabolite MTT value also showed a trend to increase with the lipophilicity of the parent ester (from 15.72 s for C3SA to 61.97 s for C8SA). However, the parent MTT value shows no significant change across the series.

6 The two-compartment dispersion model was used to derive the kinetic parameters for parent ester, pregenerated SA and generated SA. Consequently, these parameters were used to estimate the values of AUC′, MTT and CV^2 for the parent ester and metabolite. The moments values obtained using the two-compartment dispersion model show similar trends to the corresponding moments values obtained from the outflow profiles using a non-parametric approach.

7 The more lipophilic aspirin analogues are more confined to the portal circulation after oral administration than aspirin due to their more extensive hepatic elimination avoiding systemic prostacyclin inhibition. Given that aspirin's selectivity as an anti-thrombotic agent has been postulated to be due to selective anti-platelet effects in the portal circulation, the more lipophilic and highly extracted analogues are potentially more selective anti-thrombotic agents than aspirin.

Keywords: Aspirin analogues; hepatic extraction; lipophilicity; hepatic elimination; portal circulation; anti-thrombotic agent

Introduction

Aspirin (O-acetyl salicylic acid) is widely prescribed as an anti-thrombotic agent in cardiovascular disease. The pharmacological target of the drug is the prostaglandin synthase enzyme system which is acetylated by aspirin thus inhibiting cyclooxygenase activity (Lecomte *et al*., 1994). It has been postulated that the anti-thrombotic effect of aspirin may be maximized if the solute could be restricted to the portal circulation avoiding systemic prostacyclin inhibition (Siebert *et al*., 1983; Pedersen *et al*., 1984; Roberts *et al*., 1986; McLeod *et al*., 1988). Confining the inhibition of cyclooxygenase activity to the portal circulation depends on extensive hepatic extraction of the solute. The extraction of aspirin by the perfused rat liver has been shown to be moderate, with a hepatic availability for aspirin (the fraction of the aspirin dose surviving a single pass of the liver) equal to 0.73 at a perfusion flow rate of 30 mL/min (Mellick and Roberts, 1996). The homologous series of O-acyl derivatives of salicylic acid (Figure 1) are analogous compounds to aspirin. We hypothesized that the hepatic extraction of a more lipophilic aspirin analogues may be greater than that of aspirin. It was proposed that an enhanced hepatic extraction of certain aspirin analogues would make them potential anti-thrombotic agents through their selectivity in platelet cyclooxygenase inhibition in the portal circulation.

When a drug is injected as a bolus into the liver, the observed venous outflow concentration of the drug *versus* time

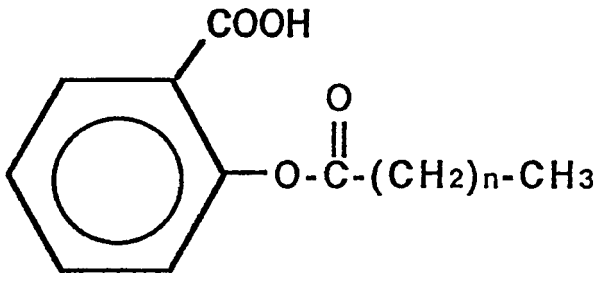

Figure 1 General structure of O-acyl ester of salicylic acid ($n=0$, C2SA; $n=1$, C3SA; $n=2$, C4SA; $n=3$, C5SA; $n=4$, C6SA; $n=6$, C8SA).

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profile can provide a valuable insight into the kinetic processes that occur during the first-pass of a drug through the liver. This technique is particularly useful when applied to the investigation of hepatic structure-pharmacokinetic relationships using an isolated perfused rat liver, where factors such as perfusate flow rate and drug binding to perfusate components can be accurately controlled and the influence of recirculation on drug elution patterns is avoided. However, only a few studies have examined the relationship between hepatic disposition and physicochemical properties within a homologous series of compounds in the perfused liver. Chou *et al.* (1993;1995) have reported relationships between the dispersion, distribution and lipophilicity for a series of barbiturates.

The present paper investigates the relative hepatic extraction of salicylic acid and its O-acyl congeners (acetyl, propionyl, butanoyl, pentanoyl, hexanoyl and octanoyl) using the *in-situ* perfused rat liver preparation. The relationship between lipophilicity and hepatic disposition of the parent esters and metabolite are examined using non-parametric statistical moments analysis and the dispersion model. The lipophilicity and some other physicochemical properties of these aspirin analogues have been reported elsewhere (Table 1) (Hung *et al.*, 1997).

Hepatic metabolite kinetics following administration of a parent solute into the perfused liver are not well defined (Pang *et al.*, 1995). Moreover, the effect of parent compound structure on the hepatic pharmacokinetics of a common metabolite in the perfused liver has not been reported. However, this information is extremely important in drug design, particularly if the administered compound is a prodrug or if metabolites are potentially toxic. This is, as far as we are aware, the first attempt to examine the relationship between parent compound physicochemical structure to metabolite kinetics in a homologous series. This paper is a practical example of the theory outlined in Mellick *et al.* (1998) showing that by knowing the kinetic properties of parent compound and pregenerated metabolite, we can adequately predict generated metabolite disposition in the liver.

Theory

Two-compartment dispersion model (Figure 2)

The outflow concentration for each ester was normalized by dividing by the dose injected. The resulting outflow concentration-time profiles were then analysed using the two-compartment dispersion model with correction for catheter effects (Yano *et al.*, 1990; Evans *et al.*, 1993; Hussein *et al.*, 1994).

In the two compartment dispersion model, the normalized outflow concentration *versus* time profile, C(t) from the liver is expressed as a convolution (*) of the input function I(t), catheter transfer function $f(t)_{cath}$ and liver transfer function $f(t)_{liver}$:

$$C(t)_{total} = I(t)^{*}f(t)_{cath}^{*}f(t)_{liver} \quad (1)$$

Expressing Equation (1) in the Laplace domain with a bolus input for I(t) yields:

$$C(s)_{total} = f(s)_{cath} \cdot f(s)_{liver} \quad (2)$$

A single Gaussian distribution function was used to define $f(s)_{cath}$ and the parameters (V_{cath}, $D_{N\,cath}$) derived from the non-linear regression of catheter outflow fraction-time data were used as fixed parameters in $f(s)_{cath}$ in Equation (2):

$$f(s)_{cath} = \exp\left[\frac{1-\sqrt{1+\frac{4D_{N\,cath}V_{cath}s}{Q}}}{2D_{N\,cath}}\right], \quad (3)$$

where V_{cath} and $D_{N\,cath}$ are the volume of distribution for the catheters and the dispersion number for the catheter as defined by the catheter outflow-time profiles, Q is the perfusate outflow rate.

According to the two-compartment dispersion model the Laplace expression describing the outflow profile of an exchanging solute in the liver is given by the following Equation:

$$f(s)_{liver} = \exp\left[\frac{1-\sqrt{1+\frac{4D_N V_E[k_1+s-\frac{k_1k_2}{s+k_2+k_{el}}]}{Q}}}{2D_N}\right], \quad (4)$$

where V_E and D_N are the volume of distribution in the extracellular space and the dispersion number which describes

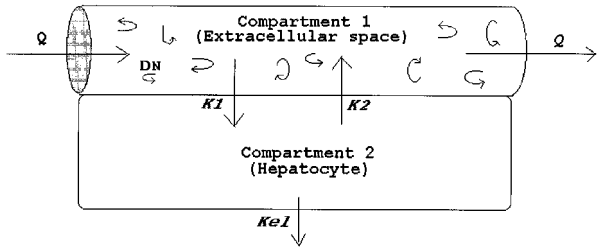

Figure 2 Schematic Overview: Two-compartment dispersion model. V_E is the volume of compartment one (which physiologically represents the combined volumes of the vascular and Disse spaces in the liver). V_C is the volume of compartment 2 (which physiologically represents the volume of the cellular space). D_N is the dispersion number representing the relative dispersion of solute in the compartment one. Q is the perfusion flow rate. k_1, k_2 and k_{el} representing the influx, efflux and elimination rate constant, respectively.

Table 1 The O-acyl derivatives of salicyclic acid (SA) and their physicochemical properties (Hung *et al.*, 1997): molecular weight, aqueous solubility, octanol/water partition coefficient, spontaneous hydrolysis in protein free perfusate at 25° and 37°C and melting point

Derivative	*Abbreviation*	*MW*[a]	*S*[b] (mM) 25°C	*log PC*[c] 25°C	$t_{1/2}$[d] 25°C (h)	$F_{5\ min}$[e] 37°C	*MP*[f] (°C)
O-acetyl SA (aspirin)	C2SA	180	18.5	1.19	40.53	0.98	136
O-propionyl SA	C3SA	194	6.3	1.72	47.14	0.99	91
O-butanoyl SA	C4SA	208	2.7	2.25	141.43	0.99	80
O-pentanoyl SA	C5SA	222	0.33	2.78	113.61	0.99	81
O-hexanoyl SA	C6SA	236	0.08	3.31	117.46	0.98	74
O-octanoyl SA	C8SA	264	0.008	4.37	135.88	0.99	77

[a]Molecular weight; [b]aqueous solubility; [c]octanol/water partition coefficient; [d]half life (spontaneous hydrolysis); [e]fraction remaining after 5 min at 37°C (unpublished data); [f]melting point.

the spread of reference marker residence times in the liver following bolus input, s is the Laplace variable, k_1 and k_2 representing the first order influx and efflux rate constants across the hepatocyte membrane and k_{el} is the hepatocyte's first order elimination rate constant (all with units of time^{-1}). The rate constants k_1, k_2 and k_{el} are frequently expressed in the following physiological terms:

$$k_1 = \frac{PSf_u}{V_E}, k_2 = \frac{PSf_{uc}}{V_C}, k_{el} = \frac{CL_{\text{int}} f_{uc}}{V_C} \tag{5}$$

where V_C are the volume of hepatocytes. f_u and f_{uc} are the fraction of solute unbound in the perfusate and the cells, respectively. PS is the permeability-surface area product (with units of volume per time) of the hepatocyte membrane to the solute and CL_{int} is the intrinsic clearance of the liver (defined as $CL_{int} = \sum_{i=1}^{n} \frac{V_{m,i}}{Km,i}$) (Gillete. 1971). Since f_u equalled to unity (protein-free perfusate was used in the present work) and V_C/f_{uc} can be presented as V_T (apparent tissue distribution volume), the rate constants k_1, k_2 and k_{el} can also be expressed as $\frac{PS}{V_E}, \frac{PS}{V_T}$, and $\frac{CL_{\text{int}}}{V_T}$, respectively (Weiss *et al*., 1997).

The Laplace transformation of the outflow concentration for a generated metabolite, $\hat{c}_m$, after bolus input of parent drug can be determined by the following equations (Mellick *et al*., 1998):

$$\hat{c}_m = \frac{k_{elp} k_{1p} k_{2m} (IG_1 - IG_2)}{g_{1m} g_{2p} - g_{1p} g_{2m}} \frac{Dp}{Q} \tag{6}$$

where Dp is the dose of parent ester administered, subscript p and m are used to denote parameters for a parent compound and metabolite, respectively.

$$IG_1 = \exp\left[\frac{1 - \sqrt{1 + \frac{4D_N V_E g_{1p}}{Q g_{2p}}}}{2D_N}\right], \tag{7}$$

$$IG_2 = \exp\left[\frac{1 - \sqrt{1 + \frac{4D_N V_E g_{1m}}{Q g_{2m}}}}{2D_N}\right], \tag{8}$$

$$g_{1p} = k_{elp}\ k_{1p} + s\left(k_{1p} + k_{2p} + k_{elp}\right) + s^2, \tag{9}$$

$$g_{2p} = k_{elp} + k_{2p} + s, \tag{10}$$

$$g_{1m} = k_{elm}\ k_{1m} + s\left(k_{1m} + k_{2m} + k_{elm}\right) + s^2, \tag{11}$$

$$g_{2m} = k_{elm} + k_{2m} + s. \tag{12}$$

Parametric moments analysis using the dispersion model

A model interpretation of the moments obtained from impulse response studies in isolated organ is provided by the dispersion model (Roberts *et al*., 1990):

$$F = \exp\left(\frac{1 - a}{2D_N}\right), \tag{13}$$

where

$$a = \sqrt{1 + 4\ R_N\ D_N}, \tag{14}$$

D_N is equal to half the normalized variance for a non-extracted reference indicator ($\frac{CV^2}{2}$) for the dispersion model with mixed boundary conditions and R_N is the efficiency number which characterizes the elimination of solute by the liver and is defined by equation (15):

$$R_N = \frac{f_u PSCL_{\text{int}}}{Q(PS + CL_{\text{int}})} \tag{15}$$

R_N is usually estimated from equation (13) and (14) using the estimated F of a solute and D_N deduced from the CV^2 for a non-extracted reference solute.

Expression for mean transit time (MTT) and normalized variance (CV^2) for a solute in the liver, as defined by the dispersion model, are shown in Equation (16) and (17) (Roberts *et al*., 1990):

$$MTT = \frac{V_E}{Qa}\left[1 + \frac{f_u V_C PS^2}{f_{uc} V_E (PS + CL_{\text{int}})^2}\right] \tag{16}$$

$$CV^2 = \frac{2D_N}{a} + \frac{V_C^2\ PS^2\ f_u\ f_{uc}\ Q(PS + CL_{\text{int}})}{[aV_E\ f_{uc}(PS + CL_{\text{int}})^2 + V_C\ f_u\ PS^2]^2} \tag{17}$$

The area under the curve corrected for dose injected (AUC_p'), MTT_p and CV^2_p for parent compound can be obtained from the two-compartment dispersion model parameter estimates by rearranging Equation (5) and substitutes to Equations (4), (16), and (17), to give,

$$AUC_p' = \frac{Q}{D_p}\hat{c}_p(s=0) = \exp\left[\frac{1 - \sqrt{1 + \frac{4D_N V_E\left[k_{1p} - \frac{k_{1p}k_{2p}}{k_{2p}+k_{elp}}\right]}{Q}}}{2D_N}\right], \tag{18}$$

$$MTT_p = \frac{d\ln(\hat{c_p})}{ds}(s=0) = \frac{V_E}{aQ}\left(1 + \frac{k_{1p}k_{2p}}{{k_{2p}}^2 + 2k_{2p}k_{elp} + {k_{elp}}^2}\right), \tag{19}$$

$$CV^2_p = \frac{d^2\ln(\hat{c_p})}{ds^2}(s=0) = \frac{2D_N}{a} + \frac{2aV_c{k_{2p}}^2 f_u f_{uc} Q\left(k_{2p} + k_{elp}\right)}{\left[f_u V_E\left(k_{2p} + k_{elp}\right)^2 + f_u\ {k_{2p}}^2 V_c\right]^2}, \tag{20}$$

where $\hat{c}_p$ is the Laplace transform of concentration for the parent compound.

This allows an estimation of the moments for a parent compound (AUC'_p, MTT_p, and CV^2_p) using the solute parameter values obtained from the dispersion model fitting.

Equations (6) to (12) can be expressed as Equation (21):

$$\hat{c}_m = \frac{k_{2m}\ k_{1p}\ k_{elp}}{g_{1m}g_{2p} - g_{1p}g_{2m}}\left(\hat{c}_p - \frac{\hat{c}_{mp}\ D_p}{D_{mp}}\right), \tag{21}$$

where $\hat{c}_{mp}$ is the Laplace transform of concentration for the metabolite introduced as parent (pregenerated metabolite) and D_{mp} is the dose of pregenerated metabolite. It is possible to find expressions for the moments of metabolite (AUC'_m, MTT_m and CV^2_m) using Equation (21) (Mellick *et al*., 1998). The area under the curve corrected for dose of parent compound (AUC'_m), mean transit time (MTT_m) and normalized variance (CV_m^2) of metabolite can be predicted from Equations (22) to (25):

$$AUC_m' = \hat{c}_m(s=0) = \frac{k_{2m}\ R_{Np}}{(k_{2m} + k_{elm})(R_{Nm} - R_{Np})}(AUC_p' - AUC'^{mp}), \tag{22}$$

where

$$R_{Nz} = \frac{g_{1z}(s=0)}{g_{2z}(s=0)} = \frac{k_{1z}k_{elz}}{k_{2z}+k_{elz}} \cdot \frac{V_E}{Q} \tag{23}$$

subscript z is p for parent compound or m for metabolite,

$$MTT_m = -\frac{\hat{c}_m'(s=0)}{AUC_m'} = \frac{MTT_p AUC_p' - MTT_{mp} AUC_{mp}'}{AUC_p' - AUC_{mp}'} + \frac{1}{R_{Nm}-R_{Np}}\left(\frac{k_{1m}\frac{V_E}{Q}-R_{Np}}{k_{2m}+k_{elm}} - \frac{k_{1p}\frac{V_E}{Q}-R_{Nm}}{k_{2p}+k_{elp}}\right) \tag{24}$$

$$CV_m^2 = \frac{1}{MTT_m^2}\left[\frac{CV_p{}^2 MTT_p{}^2 AUC_p' - CV_{mp}^2 MTT_{mp}^2 AUC'_{mp}}{AUC'_p - AUC_{mp}'} - \frac{AUC_p' AUC'_{mp}(MTT_p - MTT_{mp})^2}{(AUC_p' - AUC'_{mp})^2} + \frac{1}{(R_{Nmp}-R_{Np})^2}\left(\frac{k_{1m}\frac{V_E}{Q}-R_{Np}}{k_{2m}+k_{elm}} - \frac{k_{1p}\frac{V_E}{Q}-R_{Nm}}{k_{2p}+k_{elp}}\right)^2 + \left(\frac{2}{R_{Nm}-R_{Np}}\right)\frac{\frac{V_E}{Q}(k_{1p}-k_{1m})}{(k_{2p}+k_{elp})(k_{2m}+k_{elm})}\right]. \tag{25}$$

Methods

Esters synthesis and rat liver perfusions

The O-acyl derivatives of salicylic acid (Figure 1) were prepared by esterification of salicylic acid with the appropriate acyl-anhydride as described elsewhere (Hung *et al*., 1997). The single-pass *in situ* rat liver perfusion preparation used in this study is described in detail previously (Cheung *et al*., 1996). Briefly, livers were perfused with Krebs-Henseleit buffer (pH 7.4) at 30 mL/min. A saturated solution (volume 50 μL) of the particular O-acyl ester of salicylic acid in Krebs-Henseleit buffer containing [^{14}C]-salicylic acid (1 μCi, 3 μg) was injected into the liver via the portal vein catheter. Outlet samples were collected via a fraction collector over 1 min. The outlet samples were assayed for [^{14}C]-salicylic acid (scintillation counting), O-acyl ester and metabolite SA (high performance liquid chromatography, HPLC analysis) (Hung *et al*., 1998).

Injections of C4SA and C5SA were performed in each of four liver preparations. C3SA injections were made in a separate series of four preparations. In two livers, the uptake and metabolism of C6SA and C8SA were investigated. The order of injections was randomized in each preparation. All data for C2SA (aspirin) was taken from previous studies in our laboratory (Mellick and Roberts, 1992; 1996).

Modelling of the outflow concentration-time profiles

The outflow fraction-time data for pregenerated SA ([^{14}C]-salicylic acid) and the parent esters were fitted to the two-compartment dispersion model with correction for catheter effects using Equations (2), (3), and (4) (Yano *et al*., 1990; Evans *et al*., 1993; Hussein *et al*., 1994) to obtain influx, efflux, elimination rate constants. In all cases, the pregenerated SA profile was fitted first and the D_N, V_E values obtained were used in the fitting of the corresponding parent ester curve from the same injection. To fit the generated metabolite profile, the D_N, V_E, k_{1p}, k_{2p}, and k_{elp} values were fixed and Equation (6) was fitted to the data to determine k_{1m}, k_{2m}, and k_{elm} values. All fitting was performed using the program Scientist (MicroMath Scientific Software, Salt Lake City, UT) with data weighted ($1/y_{obs}{}^2$) in all cases.

Non-parametric moments analysis

The area under the curve (AUC′), mean transit time (MTT) and normalized variance (CV^2) for parent ester and generated metabolite (SA) were determined directly from the outflow-concentration profiles using the non-parametric parabolas-through-the-origin (PTTO) method (extrapolated to infinity) with tail correction (Purves, 1994) and equations (26) to (29).

$$\mathrm{AUC}' = \frac{Q.AUC}{D_p}, \tag{26}$$

$$\mathrm{MTT} = \frac{AUMC}{\mathrm{AUC}}, \tag{27}$$

$$\mathrm{CV}^2 = \frac{\sigma^2}{MTT^2}, \tag{28}$$

$$\sigma^2 = \frac{\int_0^\infty t^2 C(t)dt}{\int_0^\infty C(t)dt} - MTT^2, \tag{29}$$

where D_p is the dose of parent ester administered, AUMC is the area under the first moment curve and C(t) is the metabolite's outflow concentration *versus* time profile, AUC′ is the area under the curve normalized for flow rate and the dose of the parent solute (in molar equivalents). For parent ester, AUC′ is equivalent to the hepatic availability (F) which represents the fraction of the injected dose which escapes removal across the liver.

Estimation of statistical moments using the dispersion model

The influx, efflux and elimination parameters for parent ester and pregenerated metabolite, obtained through fitting the outflow profiles to the two-compartment dispersion model, were substituted into equations (18) to (20) and (22) to (25) to obtain estimations for AUC′, MTT and CV^2 for the parent esters and the generated metabolites.

All data is presented as mean $\pm$ standard deviation unless otherwise stated. A $P<0.05$ was taken as significant.

Results

Figure 3 shows the logarithm of normalized outflow concentration *versus* time data and the dispersion model regression lines for the various parent esters, the pregenerated metabolite ([^{14}C]-salicylic acid), and generated metabolite (salicylic acid). The pregenerated metabolite outflow curves remain constant for the various ester injections and is in good agreement with those previously determined for [^{14}C]-salicylic acid (Hussein *et al*., 1994; Mellick and Roberts, 1996). The estimated two-compartment dispersion model parameters for pregenerated metabolite ([^{14}C]-salicylic acid) were $D_N = 0.09 \pm 0.08$, $k_1 = 0.32 \pm 0.12$ s^{-1}, $k_2 = 0.11 \pm 0.04$ s^{-1}, $k_{el} = 0.01 \pm 0.01$ s^{-1} and $V_E = 0.41 \pm 0.16$ mL g^{-1} liver ($n=20$). They are comparable with the results of Hussein *et al*. (1994) ($D_N = 0.08 \pm 0.03$, $k_1 = 0.56 \pm 0.04$ s^{-1}, $k_2 = 0.10 \pm 0.01$ s^{-1}). Figure 3 also shows that the generated SA curve becomes closer to the pregenerated [^{14}C]-SA curve as the carbon number in the parent ester O-acyl moiety increased.

Table 2 lists the kinetic parameters obtained from these fittings. It shows that the influx (k_{1p}) and elimination (k_{elp}) rate coefficients for the parent ester increase directly with the lipophilicity ($r^2 = 0.98$ for k_{1p} and $r^2 = 0.87$ for k_{elp}), whereas the efflux rate coefficient (k_{2p}) for the parent ester decreases directly with the lipophilicity ($r^2 = 0.99$). The influx rate coefficient (k_1), efflux rate coefficient (k_2) and elimination rate coefficient (k_{el}) of salicylic acid were similar regardless of whether they were obtained from the pregenerated [^{14}C]-SA curves or generated SA curves from any parent ester. The values of permeability-surface area product (PS), apparent tissue distribution volume (V_r) and ratio of the apparent tissue distribution volume to the extracellular volume (V_T/V_E) for the parent esters increase directly with the lipophilicity ($r^2 = 0.97$

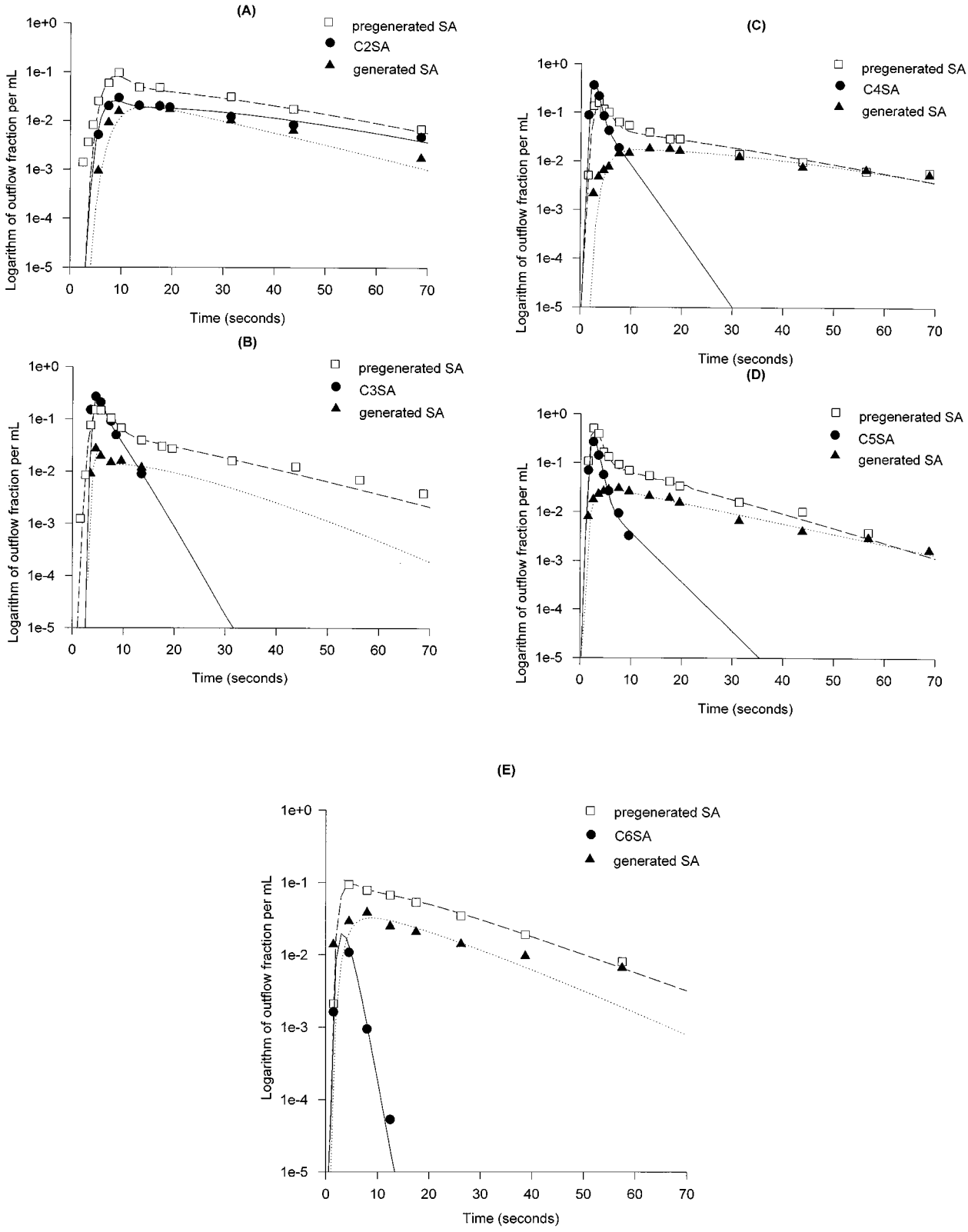

Figure 3 Typical outflow profiles for the O-acyl esters, pregenerated SA, and metabolite SA (data weighted, $1/y_{obs}^2$) in the regressions. (A) C2SA; (B) C3SA; (C) C4SA; (D) C5SA; (E) C6SA.

Table 2 Kinetic parameters derived from two-compartment dispersion model fitting for O-acyl esters of salicyclic acid and salicyclic acid (metabolite) generated from corresponding parent esters

	Influx rate coefficient (k_1) (s^{-1})		*Efflux rate coefficient* (k_2) (s^{-1})		*Elimination rate coefficient* (k_{el}) (s^{-1})		*Permeability-surface area product* (PS) (mL min^{-1} g^{-1} liver)		*Tissue distribution volume* (V_T) (mL g^{-1} liver)		*Ratio of tissue distribution volume to extracellular volume* (V_T/V_E)	
Compound	*Parent* (k_{1p})	*Metabolite* (k_{1m})	*Parent* (k_{2p})	*Metabolite* (k_{2m})	*Parent* (k_{elp})	*Metabolite* (k_{elm})	*Parent*	*Metabolite*	*Parent*	*Metabolite*	*Parent*	*Metabolite*
C2SA[a]	0.09±0.02	0.33±0.14	0.22±0.14	0.08±0.06	0.13±0.06	0.04±0.04	2.21±0.04	8.12±0.14	0.17±0.02	1.69±0.18	0.41	4.13
C3SA[a]	0.28±0.02	0.40±0.18	0.21±0.10	0.10±0.04	0.28±0.02	0.02±0.02	6.89±0.04	9.84±0.44	0.55±0.02	1.64±0.36	1.33	4.00
C4SA[a]	0.41±0.06	0.41±0.12	0.10±0.08	0.09±0.04	0.31±0.02	0.03±0.02	10.09±0.14	10.09±0.28	1.68±0.06	1.87±0.24	4.10	4.56
C5SA[a]	0.52±0.08	0.38±0.06	0.05±0.02	0.09±0.04	0.41±0.02	0.02±0.02	12.79±0.20	9.35±0.14	4.26±0.32	1.73±0.12	10.40	4.22
C6SA[a]	0.82	0.35	0.02	0.07	0.46	0.01	20.17	8.61	16.81	2.05	41.00	5.00

[a] Mean±s.d. ($n=4$) [b] Mean ($n=2$).

Table 3 Non-parametric moments calculator results and predictions using dispersion model of hepatic elimination for O-acyl esters of salicyclic acid and salicyclic acid generated from corresponding parent esters

	AUC				*MTT (s)*				CV^2			
	Parent		*Metabolite*		*Parent*		*Metabolite*		*Parent*		*Metabolite*	
Compound	*moments*	*prediction*	*moments*	*prediction*	*moments*	*prediction*	*moments*	*prediction*	*moments*	*prediction*	*moments*	*prediction*
C2SA[a]	0.85±0.12	0.73±0.02	0.12±0.04	0.27±0.02	5.58±0.72	6.52±3.02	20.12±5.40	14.53±9.68	0.33±0.18	0.53±0.06	0.73±0.20	0.31±0.22
C3SA[b]	0.53±0.10	0.54±0.12	0.12±0.08	0.28±0.12	5.54±0.84	5.53±3.60	15.72±10.2	18.95±6.42	0.16±0.08	0.23±0.04	0.32±0.30	0.41±0.14
C4SA[b]	0.40±0.14	0.35±0.16	0.40±0.14	0.55±0.10	6.03±3.00	5.00±2.60	40.65±8.36	27.76±5.88	0.18±0.04	0.17±0.02	0.76±0.12	0.26±0.22
C5SA[b]	0.26±0.04	0.28±0.06	0.59±0.32	0.68±0.02	4.45±0.92	3.13±0.80	31.09±8.32	27.99±4.62	0.13±0.06	0.15±0.06	0.71±0.24	0.43±0.34
C6SA[c]	0.02	0.03	0.63	0.97	4.58	3.28	49.94	38.33	0.18	0.17	1.07	0.47
C8SA[c]	n.d.[d]	n.d.	0.95	n.d.	n.d.	n.d.	61.97	n.d.	n.d.	n.d.	1.05	n.d.

[a]From Mellick *et al.*, 1992; 1996; [b]Mean±s.d. ($n=4$); [c]Mean ($n=2$); [d]Not determined.

for PS, $r^2 = 0.71$ for V_T and V_T/V_E), whereas these values for metabolite obtained from different parent esters remain relatively constant.

Table 3 lists the results of non-parametric moments analysis for the parent esters and SA generated from corresponding parent esters' bolus studies. It shows that the metabolite hepatic AUC′ value (AUC'_m) increases directly with the lipophilicity of the parent ester ($r^2 = 0.93$), from 0.12 for C2SA to 0.95 for C8SA. By contrast, there is an inverse linear relationship ($r^2 = 0.97$) between the lipophilicity and the hepatic AUC′ value (AUC'_p) of the parent ester, with the value decreasing from 0.85 for C2SA to zero for C8SA. On average 89% of the ester dose was recovered as either parent ester or metabolite SA. This total recovery was independent of ester carbon number. The metabolite MTT value (MTT_m) shows a trend to increase with the lipophilicity of the parent ester (from 15.72 s for C3SA to 61.97 s for C8SA, $r^2 = 0.85$). On the contrary, the parent MTT value (MTT_p) shows no significant change across the series. Both the parent and metabolite CV^2_s (CV^2_p and CV^2_m) showed no significant difference for all the esters. Table 3 also presents the two-compartment dispersion model predictions of the parent compound and metabolite statistical moments values using Equations (18) to (20) and (22) to (25). The predicted values are similar to the corresponding moments values obtained from the outflow profiles using a non-parametric approach.

Discussion

The present paper has shown that phenolic esters with a longer carbon chain length in the O-acyl moiety have higher hepatic extraction. Siebert *et al.* (1983), Pedersen *et al.* (1984) and Roberts *et al.* (1986) have postulated a high first pass for aspirin and its platelet inhibition in the portal circulation as the basis for its selectivity on thromboxane A_2 production. If these more lipophilic aspirin analogues show anti-thrombotic activity, these esters may be potential anti-thrombotic agents in that the extensive first pass metabolism may enable selective platelet acylation and inhibition of thromboxane A_2 production through inhibition of cyclooxygenase in the portal circulation. The greater hepatic extraction might reduce the exposure of the systemic circulation to these orally administered esters; thereby (*i*) minimizing prostacyclin inhibition within the vessel endothelium; and (*ii*) minimizing any re-secretion of circulating cyclooxygenase-binding drug into the stomach. Moreover, the more lipophilic drugs may be absorbed more quickly than aspirin across the gastric mucosa, thus reducing their potential for primary ulcerogenic effects.

To our knowledge, this study is the first report of hepatic metabolite kinetics across a homologous series of solutes. The O-acyl esters of salicylic acid were an ideal group for this initial study for a number of reasons. Firstly, across the series, there is a large variation in hepatic extraction (from moderate extraction for C2SA (aspirin) to almost complete extraction for C8SA). This allows a comparison of the metabolite kinetics when a small amount of the parent dose is converted to metabolite to those when the entire dose is converted to metabolite. In addition, the esters in the series are metabolised via enzymatic hydrolysis to a common metabolite, SA, which is not appreciably metabolised across the liver, under the conditions of the study (Hussein *et al.*, 1994; Mellick & Roberts, 1996). This is a special case, since usually, there are parallel and sequential metabolic pathways for solutes which need to be considered. We have shown that non-enzymatic hydrolysis was minimal under our experimental protocol (Hung *et al.*, 1997) and that almost the total dose (average of 89%) of ester could be recovered as either parent ester or SA in the liver's outflow.

The study shows that the dispersion model can be used to describe the metabolite outflow profile following bolus injection of parent compound. It was found that the metabolite kinetic parameters obtained from a fit of the metabolite profile (Table 2) were in good agreement with those obtained from the fitting of preformed [^{14}C]-SA, administered as a parent compound. This suggests that given knowledge of the distribution characteristics of a parent compound and a metabolite administered as a parent compound, it is possible to predict the outflow curve of a metabolite formed within the liver. This is potentially useful when designing prodrugs for delivery of a metabolite.

It should be noted that the dispersion model is based on simplifying assumptions regarding the slope of the extracellular reference curve (single inverse Gaussian), which may lead to biased parameter estimates (Weiss *et al.*, 1997). However, in the present case this effect can be neglected for the parent compounds, because of the permeability-limited elimination. The dispersion model for metabolite kinetics developed by this group appears to be the only model able to describe precursor-metabolite kinetics using nonlinear regression and accounting for both catheter effects and precursor disposition. In theory, the form of analysis should be model independent as this work is limited to linear systems and no other model is available for metabolite kinetics.

We have attempted to use three structural models: (1) a stochastic model of drug transport (Weiss *et al.*, 1997), (2) the two-compartment dispersion model (Evans *et al.*, 1993; Hussein *et al.*, 1994) and (3) the Goresky model (Goresky *et al.*, 1992) to estimate influx, efflux, and elimination rate constants for the parent esters in this homologous series of O-acyl salicylates. Each relied on fitting an extracellular reference solute, [^{3}H]-sucrose. We found that the best fit of the parent ester data using these methods was with the simplified model where $k_{2p} = 0$. Thus, these methods cannot be used to estimate some important kinetic parameters (eg V_T and V_T/V_E). We solved this problem by including a tracer amount of [^{14}C]-SA instead of [^{3}H]-sucrose in the bolus and fitting this pregenerated SA outflow profile following the method of Hussein *et al.* (1994) to obtain D_N and V_E values. These values were subsequently used to fit the parent ester curves. This method proved robust and superior to the previous methods that obtained D_N and V_E from fitting the sucrose outflow profile. One reason for this may be that the V_E exhibited by [^{14}C]-SA (0.41 ± 0.16 mL g^{-1} liver) may more closely represent the distribution volume of the parent esters than the extracellular volume exhibited by sucrose (0.31 ± 0.08 mL g^{-1} liver). Hussein *et al.* (1994) showed that the D_N values for water differed significantly from those of SA. They suggested that the consistently lower D_N obtained for SA may result from the very rapid flux of [^{14}C]-SA and limitations of the collection conditions used (Hussein *et al.*, 1994). This may also be true in this study. It is generally considered that D_N is a physical value, characteristic of the organ and independent of the solute, reflecting the anatomy of the organ (Chou *et al.*, 1993; 1995; Hussein *et al.*, 1994).

Statistical moments are frequently used to describe the disposition of solutes in the liver (Roberts *et al.*, 1990; Hussein *et al.*, 1994; Mellick & Roberts, 1996). It has recently been shown that metabolite statistical moments can be predicted using various models of hepatic elimination (Mellick *et al.*, 1998). In this paper we present the equations for metabolite moments in terms of the parent compound and preformed

metabolite dispersion model coefficients, and show that the estimates of the moments are similar to the non-parametric statistical moments obtained directly from the outflow profiles (Table 3). This was done to show how changes in the influx, efflux and elimination coefficients affect the statistical moments of the metabolite outflow curve.

The metabolite AUC′ value increased directly with the lipophilicity of the parent ester. This mirrors the decrease in parent compound AUC′ (increase in parent compound extraction) with lipophilicity, and reflects the increased permeability − surface area product (PS) and CL_{int}. The intrinsic clearance for each parent ester can be obtained from the two-compartment dispersion model parameter estimates by substituting and rearranging Equations (5) and (15):

$$CL_{int} = \frac{k_{1p}k_{elp}V_E}{f_u k_{2p}.} \quad (30)$$

Figure 4 shows that both the metabolite AUC′ value and intrinsic clearance (CL_{int}) of these esters increase with the lipophilicity of the parent ester.

There is a trend to higher metabolite MTT with increasing lipophilicity of parent compound (15.72 s for propionyl and 61.97 s for octanoyl). This mirrors the increase in the ratio of the apparent tissue distribution volume to the extracellular volume (V_T/V_E) for the parent compound with lipophilicity. However, metabolite MTT and CV^2 are convoluted parameters (Equations (24) and (25)) which may be altered by small changes in parent compound or metabolite influx, efflux or elimination coefficients. The presented equations allow consideration of how the moments will be changed when the kinetic parameters of the parent compound or metabolite are varied. This may be of particular importance if there is a desire to maximize the organ MTT of a metabolite which is pharmacologically active, or minimize the MTT of a toxic metabolite.

We have examined the hepatic disposition and metabolite kinetics across a homologous series of O-acyl esters of salicylate. The outflow curves of parent esters and metabolite were well described by the two-compartment dispersion model. The influx, efflux and elimination coefficients of generated metabolite (SA) similar throughout the series and consistent with those of pregenerated metabolite ([^{14}C]-SA). The values of permeability-surface area product (PS), apparent tissue distribution volume (V_T) and ratio of the apparent tissue distribution volume to the extracellular volume (V_T/V_E) for the parent esters increase directly with the lipophilicity, whereas

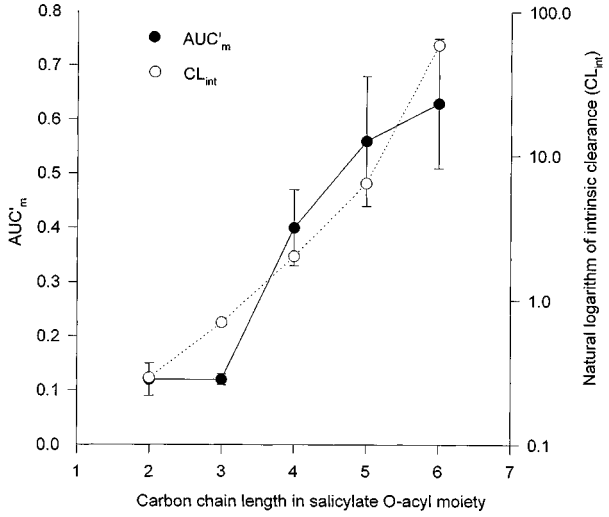

Figure 4 The relationships of the metabolite AUC′ value (AUC'_m), the intrinsic clearance (CL_{int}) of the parent ester, and carbon chain length for the O-acyl esters of salicylic acid. The figure shows that the metabolite AUC'_m and the intrinsic clearance (CL_{int}) of the parent ester followed a similar trend to increase with the lipophilicity.

these values for metabolite obtained from different parent esters remain relatively constant. The metabolite AUC′ value (AUC'_m) increased directly with the lipophilicity of the parent ester, with the value increasing from 0.12 for C2SA to 0.95 for C8SA. This mirrors the inverse linear relationship between the lipophilicity and the parent AUC′ value which decreased from 0.85 for C2SA to zero for C8SA. These more lipophilic aspirin analogues are more confined to the portal circulation after oral administration than aspirin due to their more extensive hepatic elimination avoiding systemic prostacyclin inhibition. Given that aspirin's selectivity as an anti-thrombotic agent has been postulated to be due to selective anti-platelet effects in the portal circulation, the more lipophilic and highly extracted analogues are potentially more selective anti-thrombotic agents than aspirin.

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