ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 46 (1998) 293–297

European Journal of Pharmaceutics and Biopharmaceutics

Research paper

Hepatic kinetics of SCP-1 (*N*-[α-(1,2-benzisothiazol-3(2*H*)-ona-1,1-dioxide-2-yl)-acetyl]-*p*-aminophenol) compared with acetaminophen in isolated rat liver

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Received 15 August 1997; accepted 24 April 1998

Abstract

The hepatic disposition of a new analgesic, SCP-1, a derivative of acetaminophen, was studied in the isolated perfused rat liver using a recirculating system. The aim of this study was to compare the kinetic parameters of this molecule with those of acetaminophen. Sprague–Dawley rat (230–330 g) livers were perfused for 2 h with 250 ml Krebs–Henseleit bicarbonate buffer containing SCP-1 or acetaminophen, 0.07 mmol l^{-1} ($n = 4$), 0.28 mmol l^{-1} ($n = 4$), and 0.8 mmol l^{-1} ($n = 4$) (approximately one, four and ten times the therapeutic doses in man, respectively). Perfusate samples were collected from the efflux at various times. The SCP-1 and acetaminophen perfusate concentrations were assayed by a HPLC method. Pharmacokinetic analysis was carried out using a computer program. There were significant differences between the hepatic kinetics of SCP-1 and those of acetaminophen. Thus, SCP-1 elimination half-life (mean 14.8 ± 10.0 min) was shorter than that of the acetaminophen (186.1 ± 27.7 min) ($t = 11.6$, $P = 0.0001$). While the half-life of SCP-1 increases with concentration, the half-life of acetaminophen remains constant as the concentration increases. The hepatic clearance was higher for SCP-1 than acetaminophen (mean 19.01 ± 14.5 ml min^{-1} vs. 1.29 ± 0.08 ml min^{-1}, respectively) ($t = 2.44$, $P < 0.05$), and it behaved according to dose-dependent kinetics. The SCP-1 extraction ratio was higher (mean 0.63 ± 0.49) than for acetaminophen (0.04 ± 0.01) ($t = 2.41$, $P < 0.05$) and this parameter tended to decrease as the perfusate concentrations of SCP-1 increased. It was concluded that the hepatic kinetics of SCP-1 behaved according to dose-dependent kinetics, and statistically significant differences were found between pharmacokinetics parameters of both drugs studied.

Keywords: SCP-1; Liver; Kinetics; Perfused rat liver; Pharmacokinetics; Acetaminophen

1. Introduction

The synthesis of new drugs with analgesic properties is a field of interest in therapy because the analgesics currently in use have a high incidence of adverse reactions. Hence, the search for new safer analgesics is a challenge to organic chemists for the next century. A new analgesic, a derivative of acetaminophen, named SCP-1 (*N*-[α-1,2-benzisothiazol-3(2*H*)-ona-1,1-dioxide-2-yl)-acetyl]-*p*-aminophenol) (Fig. 1 was developed by Bazan et al. [1]. The analgesic profile of SCP-1 appears to be similar to that of acetaminophen. In contrast to acetaminophen, SCP-1 had no antipyretic activity. Moreover, SCP-1 does not appear to be as toxic as acetaminophen. Paul and Johnson [2] studied the antipyretic activity of SCP-1 and acetaminophen in male rats and they found that dose-dependent acetaminophen reduced yeast-induced fever ($ED_{50} = 77.7$ mg kg^{-1}). However, SCP-1 had no antipyretic activity at the doses tested and it did not show the lethality observed with high doses of acetaminophen. It is not clear whether these differences are attributable to differences in the pharmacokinetics of the two

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0939-6411/98/$19.00
PII S0939-6411(98)00045-9

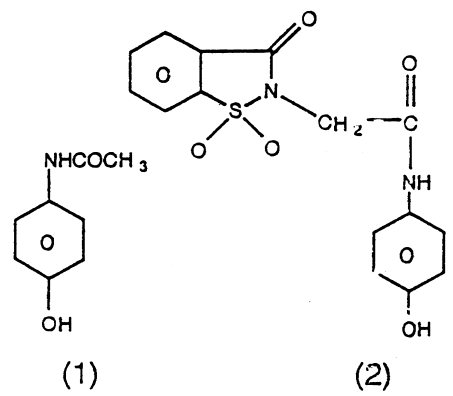

Fig. 1. Chemical structure of acetaminophen (1) and SCP-1 (2).

drugs. The lethality of acetaminophen is usually associated with the hepatotoxic effects. If toxicological testing confirms a lack of hepatotoxicity for SCP-1, this drug may be considerably more useful than acetaminophen and other NSAIDs in the treatment of chronic pain. Because SCP-1 is not an antipyretic, this drug may be useful in patients with pain but with low body temperature. Perhaps most importantly, SCP-1 could be used when analgesia is desirable, but the physician wishes to monitor body temperature to guard against infection (for example, post-operatively).

The aim of the present research was to study the hepatic pharmacokinetics of SCP-1 in comparison with acetaminophen, and to characterize the main pharmacokinetics parameters, using the isolated perfused rat liver system. No attempt was made to study the metabolism of this new analgesic.

2. Materials and methods

2.1. Chemicals

SCP-1 (99,6% purity) was synthesized by Bazan et al. [1]. All solvents were HPLC grade, and other reagents and chemicals were purchased from Merck Quimica Chilena, Santiago, Chile.

2.2. Animals

Male Sprague–Dawley rats, 230–330 g (Catholic University of Chile), were housed in well-ventilated cages and kept at room temperature at approximately 24°C.They were fed with pelleted food for 24 h before surgery, and with free access to tap water. The animals were cared for according to The Guide for the Care and Use of Laboratory Animals [3] and with ethical committee approval.

2.3. Isolated perfused livers

Rats were anesthetized with urethane (1.35 g kg^{-1}, i.p.). The abdominal cavity was opened, and the portal vein and inferior cava vein were cannulated. The liver was cut free and placed in a humidified and thermoregulated chamber. The perfusate was pumped (Masterflex, Cole Palmer, Chicago, IL, USA) through a filter, to a membrane lung (Silastic medical grade tubing, Dow Corning, Midland, MI, USA) where it was oxygenated with 95% O_2/5% CO_2, in a 37°C thermoregulated bath, to a bubble trap before reaching the liver. In the recirculating mode, the perfusate exited through the inferior vena cava cannula and dropped back into the glass reservoir before returning to the pump. The perfusate consisted of 500 ml of a modified Krebs–Henseleit bicarbonate buffer containing 1 mg ml^{-1} glucose. The perfusate was divided between two flasks each containing 250 ml. SCP-1 or acetaminophen were added to one of these flasks to a final concentration of 10 μg ml^{-1} (0.07 mmol l^{-1}), 40 μg ml^{-1} (0.27 mmol l^{-1}), or 120 μg ml^{-1} (0.80 mmol l^{-1}), corresponding to one, four and ten times the therapeutic doses in man, respectively. Livers treated in the same conditions but without drug were used as controls. The flow rate was adjusted to 3 ml $min^{-1} g^{-1}$ of liver, assuming a liver weight of 3.5% of total body weight. Constant perfusate pH was held steady at pH 7.4.

The liver was perfused for 120 min with SCP-1 or acetaminophen in the recirculating mode after an equilibrium period of 30 min in the single-pass mode with blank perfusate. For the SCP-1 and acetaminophen assay, 2 ml efflux perfusate samples were collected at 0, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min. The samples were kept frozen until assay. The viability of the liver was monitored by the rate of oxygen consumption measured, using a Cole Palmer model 5946-50 oxygen monitor (Cole Palmer, Chicago, IL, USA). During each perfusion, pH was constant at 7.4 and the oxygen consumption was higher than 25 μmol $min^{-1} g^{-1}$ liver.

It was established that SCP-1 and acetaminophen did not bind to the experimental apparatus (injector, tubing, cannulas or collection apparatus) used in the perfusion studies.

In this work, four livers were perfused for each dose level.

2.4. Assay of drugs

2.4.1. SCP-1

One ml of perfusate sample was mixed with 1 ml of 0.1 N acetic acid and 100 μl of internal standard solution of theophylline (0.625 μg ml^{-1}) and extracted by passage through a SepPak C18 cartridge (Waters, MA, USA), fitted to a Luerlock glass syringe. The cartridge was washed with 5 ml 0.1 N acetic acid. Unchanged SCP-1 were eluted with 2.0 ml of methanol. The methanol fractions were evaporated to dryness under nitrogen. The residues were dissolved in 500 μl of mobile phase (acetonitrile/acetate buffer (90:10), pH 3.5). Recovery was 85.4%. Twenty μl was injected in a Shimatzu LC-9A model pump. Chromatographic separation was achieved using a Merck LiChrospher 100 RP-18 (5 μm) in a LiChroCART 125-4 4.6 × 12.5 mm column. A Shimatzu UV-spectrophotometer set at 275 nm was used to

detect SCP-1 and theophylline. Retention times for SCP-1 and internal standard were 4.0 min and 16 min, respectively. The SCP-1 detection limit was 0.1 μg ml^{-1}. Replicate analysis of perfusate samples containing 5 μg ml^{-1} SCP-1 gave an intra-day coefficient of variation of 2.3% (n = 6, SD = 0.007).

2.4.2. Acetaminophen

One ml of perfusate sample was mixed with 1 ml of 0.1 N acetic acid and 100 μl of internal standard solution of theophylline (1.25 μg ml^{-1}) and extracted by passage through a SepPak C18 cartridge (Waters) fitted to a Luer-lock glass syringe. The cartridge was washed with 5 ml of water. Unchanged acetaminophen was eluted with 2.0 ml of methanol. The methanol fractions were evaporated to dryness under nitrogen. The residue was dissolved in 500 μl of mobile phase (methanol/water, 84:16). pH was adjusted to 3.5 with acetic acid. Recovery was 89.4%. Twenty μl was injected in a Shimatzu LC-9A model pump. Chromatographic separation was achieved using a Merck LiChrospher 100 RP-18 (5 μm) in a LiChroCART 125-4 4.6 × 12.5 mm column. A Shimatzu UV-spectrophotometer set at 254 nm was used for the detection of acetaminophen and theophylline. Retention time for acetaminophen and internal standard were 3.0 min and 6 min, respectively. The detection limit for acetaminophen was 0.1 μg ml^{-1}. Replicate analysis of perfusate samples containing 1 μg ml^{-1} acetaminophen gave an intra-day coefficient of variation of 3.6% (n = 6, SD = 0.007).

2.5. Pharmacokinetic calculations

The perfusate concentration–time data for acetaminophen and SCP-1 were fitted using a one- or two-compartment model according to the curve of perfusate concentrations versus time, which is described by the following equations:

$$C_t = C_o * e^{-k_{el}*t} \quad \text{(one-compartment model)}$$

Where C_t is the drug concentration at any time t, C_o is the concentration at zero time, and k_{el} is the elimination rate constant.

$$C_t = A * e^{-\alpha t} + B * e^{-\beta t} \quad \text{(two-compartment model)}$$

where C_t is the perfusate drug concentration at time t, and α and β are rate constants for the distribution and elimination phase, respectively. A and B are the time-zero intercepts of the extrapolated lines of the faster and the slower components.

The biexponential parameters A, α, B and β were computer-fitted to the elimination curve for four perfused livers using a computer program (SIPHAR/BASE version 4.0, Simed, Centre d'Etudes et de la Recherché en Statistique et Informatique Medicale, France).

The extraction ratio was calculated as clearance divided by perfusate flow rate.

The total clearance was calculated as dose/area under the curve (AUC).

2.6. Statistical analysis:

The parametric ANOVA test and the Student's t-test were used to compare the pharmacokinetic parameters of the two drugs studied. $P < 0.05$ was considered as statistically significant.

3. Results

Fig. 2 shows the curves of mean perfusate concentrations of SCP-1 versus time in comparison with acetaminophen. The SCP-1 curve shows a faster decay in the time than that of acetaminophen. So, 1 h after the perfusion, minimum concentrations of SCP-1 in the perfusate were observed. The SCP-1 disappearance from the perfusate decayed according to a monoexponential pattern.

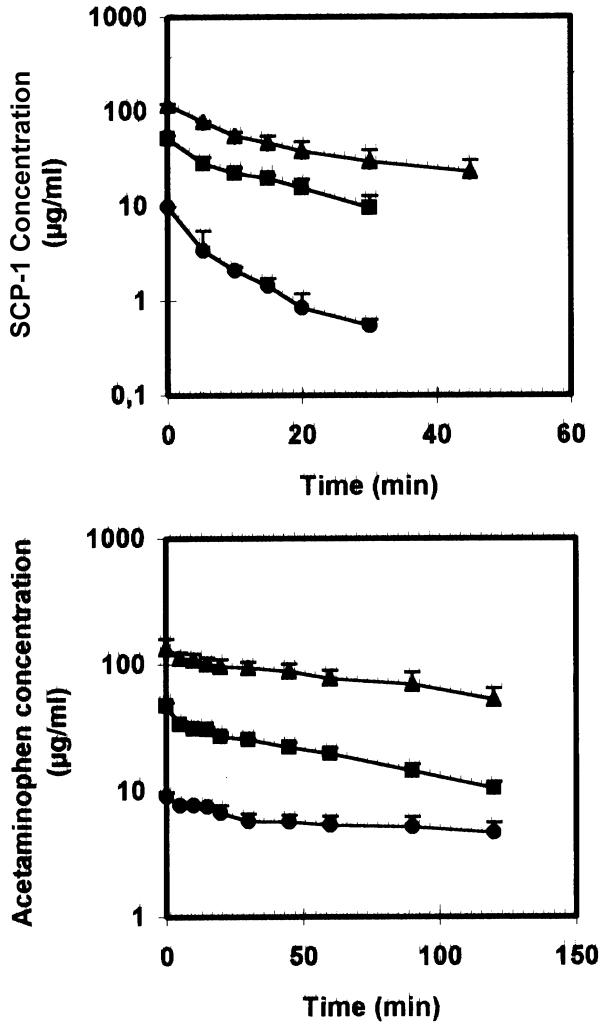

Fig. 2. Mean (±SD) of SCP-1 and acetaminophen concentrations in the perfusate vs. time for three doses studied, 10 μg ml^{-1} (●), 40 μg ml^{-1} (■), and 120 μg ml^{-1} (▲).

Tables 1 and 2 show the mean pharmacokinetics parameters of SCP-1 and acetaminophen, respectively, for the three doses studied. Statistically significant differences were observed between the main kinetic parameters of SCP-1 and acetaminophen. Thus, the half-life of elimination of the SCP-1 ranged from 5.60 to 25.46 min, depending on the drug concentration in the perfusate. For high concentrations of SCP-1 in the perfusate, the value of this parameter increased. On the other hand, the acetaminophen elimination half-life ranged from 158.39 min to 213.8 min. This difference was not statistically significant. The distribution volumes for both drugs were similar and did not present statistically significant differences (mean 277.75 ± 47.30 ml and 290.9 ± 14.81 ml, respectively; $t = 0.53$, $P < 0.60$).

Significant differences were shown for the hepatic clearance of SCP-1 for the three doses studied (35.7 ± 1.57 ml min^{-1} and 9.14 ± 2.98 ml min^{-1} for the high and low concentrations, respectively; $F = 163.0$, $P < 0.0001$). On the other hand, no change was observed in the hepatic clearance of acetaminophen when the drug concentration in the perfusate increased (mean 1.29 ± 0.08 ml min^{-1}; $F = 0.15$, $P = 0.86$).

The AUC/dose ratio obtained for SCP-1 presented a value of 28.9 ± 0.7 min ml^{-1} at 10 μg ml^{-1} concentrations, 84.40 ± 4.81 min ml^{-1} at 40 μg ml^{-1} concentrations, and 115.8 ± 31.0 min ml^{-1} at 120 μg ml^{-1}. These differences were statistically significant ($F = 23.6$, $P < 0.001$). The AUC/dose ratios for acetaminophen were similar for the three doses studied (mean 829.8 ± 15.35 min ml^{-1}) ($F = 0.02$, $P = 0.99$).

The SCP-1 extraction ratios were 1.19 ± 0.05 for the 10 μg ml^{-1} concentrations, 0.41 ± 0.07 for 40 μg ml^{-1} concentrations, and 0.30 ± 0.10 for 120 μg ml^{-1} concentrations ($F = 162.4$, $P < 0.001$). Acetaminophen presented an extraction ratio similar for the three doses studied (mean 0.04 ± 0.01; $F = 3.04$, $P = 0.1$).

4. Discussion

The concentration–time plots of SCP-1 obtained after the perfusion fitted well to a one-compartment model, and acetaminophen was better characterized by a two-compartment model. In our study, we demonstrated that the hepatic pharmacokinetics profile of SCP-1 changed with the dose used. Thus, SCP-1 concentration–time profile for the dose tenfold greater than the therapeutic dose presented significant differences in comparison with the other two doses used. The areas under the concentration vs. time curve for the three doses studied were different, indicating that at high doses SCP-1 exhibits dose-dependent kinetics of drug bioavailability, and the apparent elimination constant and the elimination half-life also change at these high doses [4]. In contrast, the kinetic parameters of acetaminophen did not change when the concentration of the drug in the perfusate increased. It is known that acetaminophen is metabolized in the liver by cytochrome *P*-450 presumably to an arylating metabolite, which is detoxified by glutathione [5–7] At high concentrations of acetaminophen, Poulsen et al. [8] did not observe changes at the pathway of drug sulfation, indicating that its metabolism is not dependent on the acetaminophen dose.

We also observed differences in the hepatic kinetics of SCP-1 compared with those of acetaminophen. Thus, clearly the average elimination half-life of SCP-1 was shorter than the average elimination half-life of acetaminophen, and this difference was statistically significant. At the three concentrations studied, the elimination half-life of SCP-1 was lower than the half-life of elimination of the acetaminophen at equivalent concentrations. The half-life was around tenfold shorter than that of acetaminophen; this represents a possible advantage, since the analgesic effect could be reached more quickly. However, the dose-dependent kinetics shown by SCP-1 could represent a disadvantage, since increasing doses or chronic medication can cause deviations from the linear pharmacokinetic profile observed with a single low dose of the same drug.

On the other hand, we found a statistically significant difference in the hepatic clearance between both drugs. Thus, at low doses of SCP-1, the hepatic clearance approaches the flow of perfusate, suggesting that this parameter is dependent on perfusate flow. This is also reflected

Table 1

Summary of pharmacokinetics parameters (±SD) of SCP-1 for three concentrations studied (according to one-compartment model)

Parameters	Perfusate concentrations (μg ml^{-1})			*P*
	10	40	120	
C_o (μg ml^{-1})	8.76 ± 1.32	44.85 ± 7.09	94.51 ± 1.82	–
k_{el} (min^{-1})	0.123 ± 0.02	0.055 ± 0.02	0.089 ± 0.03	<0.04
$t_{1/2\ el}$ (min)	5.60 ± 0.74	13.36 ± 3.59	25.46 ± 6.80	<0.005
Cl_{total} (ml min^{-1})	35.70 ± 1.57	12.19 ± 2.05	9.14 ± 2.98	<0.001
Vd_{area} (ml)	285.7 ± 38.3	226.9 ± 34.4	320.5 ± 0.9	<0.05
AUC (μg ml^{-1} min^{-1})/dose	28.9 ± 0.7	84.4 ± 4.81	115.8 ± 31.0	<0.003
Extraction ratio	1.19 ± 0.05	0.41 ± 0.07	0.30 ± 0.1	<0.0001

Table 2

Summary of pharmacokinetics parameters (± SD) of acetaminophen for three concentrations studied (according to two-compartment model)

Parameters	Perfusate concentrations (μg ml^{-1})			*P*
	10	40	120	
C_o *(A + B)* (μg ml^{-1})	9.09 ± 1.49	49.7 ± 3.8	127.4 ± 10.9	–
$\beta \times 10^{-3}$ (min^{-1})	3.2 ± 0.1	3.72 ± 1.3	4.8 ± 2.0	N.S.
$t_{1/2\ \beta}$ (min)	213.8 ± 9.6	186.1 ± 31.5	158.39 ± 53.34	N.S.
Cl_{total} (ml min^{-1})	1.20 ± 0.30	1.30 ± 0.34	1.37 ± 0.62	N.S.
Vd_β (ml)	308.0 ± 61.5	282.1 ± 39.1	282.6 ± 30.4	N.S.
AUC (μg ml^{-1} min^{-1})/dose	844.0 ± 167.6	831.8 ± 143.5	813.5 ± 304	N.S.
Extraction ratio	0.023 ± 0.004	0.043 ± 0.014	0.047 ± 0.021	N.S.

N.S., not significant.

in the extraction ratio for this compound. When a drug shows a high extraction rate, this means that the drug is removed by the liver almost as rapidly as the organ is perfused by the perfusate in which the drug is contained [4]. This characteristic was not observed with acetaminophen, which had a hepatic clearance lower than SCP-1 and a low extraction ratio. In this case, for a drug that presents these characteristics, the hepatic clearance is less affected by perfusate flow. Instead, these drugs are more affected by the intrinsic activity of the mixed-function oxidases.

5. Conclusions

In this study, we have demonstrated a significant difference in the pharmacokinetics of SCP-1 and acetaminophen. The former presented non-linear dose-dependent kinetics in comparison with the latter. The half-life of SCP-1 elimination is lower than that of acetaminophen and is dependent on the concentration of drug in the perfusate. The total clearance of the SCP-1 is dependent on drug concentration, being lower as this increases. The rate of extraction of SCP-1 is high in comparison with acetaminophen, and it is similar to the flow rate of perfusate.

Acknowledgements

Thanks to N.G. Bazan (ubazan@lsume.edu) for samples of SCP-1.

References

[1] N.G. Bazan, J. Alvarez-Builla, New *N*-acylated 4-hydroxiphenylamine derivative with analgesic properties and pharmaceutical composition containing them. Company University of Alcalá, Spain, and Louisiana State University, USA; U.S. patent 08/426,234 (1995).
[2] D. Paul, A.M. Johnson, Analgesic and Antipyretic Activity of LAU-0401, Pharmacological Report, Farmabase, Madrid, Spain, 1994.
[3] Anonymous, Governmental Activities Relating to the Use of Animals in Research. Laboratory Animal Welfare, U.S. Governmental Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training, Ilar News 28(4) (1985) 7–14.
[4] W.E. Evans, Nonlinear pharmacokinetics, in: W.E. Evans, J.J. Schentag, W.J. Jusko (Eds.), Applied Pharmacokinetics. Principles of Therapeutics Drug Monitoring, 2nd ed., Applied Therapeutics, Spokane, 1987, pp. 42–45.
[5] J.D. Jollow, S.S. Thorgeirrson, W.Z. Potter, M. Hashimato, J.R. Mitchell, Acetaminophen-induced hepatic necrosis: VI. Metabolic disposition of toxic and non-toxic doses of acetaminophen, Pharmacology 12 (1974) 251–271.
[6] J.R. Mitchell, D.J. Jollow, W.Z. Potter, D.C. Davis, J.R. Gillette, B.B. Brodie, Acetaminophen-induced hepatic necrosis: I. Role of drug metabolism, J. Pharmacol. Exp. Ther. 187 (1973) 185–194.
[7] J. Mitchell, D.J. Jollow, W.Z. Potter, D.C. Davis, J.R. Gillette, B.B. Brodie, Acetaminophen-induced hepatic necrosis: II. Role of covalent binding in vivo, J. Pharmacol. Exp. Ther. 187 (1973) 195–202.
[8] H.E. Poulsen, A. Lerche, L.T. Skovgaard, Acetaminophen metabolism by the perfused rat liver 12 hours after acetaminophen overdose, Biochem. Pharmacol. 34 (1985) 3729–3733.