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Microdialysis Study of Biliary Excretion of Chloramphenicol and its Glucuronide in the Rat

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Abstract

The biliary excretion of chloramphenicol and its glucuronide has been evaluated by use of a microdialysis probe linked to automatic on-line microbore high-performance liquid chromatography.

The microdialysis probe was inserted into the bile duct between the liver and the duodenum; to avoid obstruction of the bile duct or bile salt waste, a shunt linear probe was used. After intravenous administration of chloramphenicol succinate ($100\,mg\,kg^{-1}$) the amounts of unbound chloramphenicol and its glucuronide in the bile microdialysate were recorded. According to the pharmacokinetic results disposition of chloramphenicol seems to fit a two-compartment model whereas that of chloramphenicol glucuronide fits a one-compartment model in rat bile. The area under the concentration–time curve for chloramphenicol glucuronide was twice that for chloramphenicol in rat bile duct.

Clearance of chloramphenicol glucuronide was significantly higher than that of chloramphenicol in biliary excretion, indicating that chloramphenicol and its glucuronide are actively excreted into the bile.

Chloramphenicol has a broad spectrum antibacterial activity. It inhibits bacterial protein synthesis by blocking the transfer of soluble ribonucleic acid to ribosomes. The antibacterially inactive water-soluble form, chloramphenicol succinate, might be transformed into free chloramphenicol (the active form) by hydrolysis in the liver. The bioavailability of parenterally administered chloramphenicol succinate ranges from 55 to 95%, because the balance (from 45 to 5%, respectively) of the chloramphenicol succinate is excreted or eliminated from the body before it can be converted to the active product (Nahata & Powell 1981).

Although high-performance liquid-chromatography (HPLC) methods have already been described for determination of chloramphenicol and its metabolite in plasma (Holt et al 1995; Hummert et al 1995), we describe here a sensitive, rapid and highly selective microbore column HPLC assay with ultraviolet detection for the determination of chloramphenicol and chloramphenicol glucuronide concentrations in dialysate samples taken from rat bile. The in-vivo microdialysis technique used here also conveniently provides a clean sampling method for measurement of concentration without the need for further purification. In this study the use of an automatic on-line flow-through microdialysis probe (Scott & Lunte 1993; Hadwiger et al 1994) for bile-duct sampling coupled with a microbore-column HPLC system thus provides near real-time analysis of chloramphenicol and its glucuronide in bile dialysate samples after drug administration.

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Materials and Methods

Chemicals

Chloramphenicol (*o*-(−)-*threo*-2-dichloroacet-amido-1-[*p*-nitrophenyl]1,3-propanediol), chloramphenicol glucuronide and chloramphenicol succinate sodium

salt were purchased from Sigma (St Louis, MO). Monochloroacetic acid and reagents were obtained from Merck (Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA) was used for all preparations.

Chromatography

HPLC was performed with a BAS (West Lafayette, IN) PM-80 chromatographic pump, a Carnegie Medicine AB (Stockholm, Sweden) CMA/160 on-line injector equipped with a 10-μL sample loop, and a Soma (Tokyo, Japan) S-3702 micro UV detector. Analytes were separated on a BAS 150 mm × 1 mm i.d., 5-μm particle, RP-18 microbore column. The mobile phase was 30 : 70 (v/v) acetonitrile–10 mM monochloroacetic acid (pH 3), previously filtered through a 0·22-μm Millipore membrane. Chromatography was performed at room temperature. The UV detector was operated at 278 nm. Output data from the detector were integrated by means of an EZChrom chromatographic data system (Scientific Software, San Ramon, CA).

Experimental animals

Adult male Sprague–Dawley rats, 280–320 g, were obtained from the Laboratory Animal Centre at National Yang-Ming University (Taipei, Taiwan). These animals were pathogen free and kept in environmentally controlled quarters (24 ± 1°C and in a 12-h light–dark cycle). During experiments the rats were anaesthetized by intraperitoneal administration of sodium pentobarbital (50 mg kg^{-1}) and body temperature was maintained at 37°C by means of a heating blanket.

Use of the microdialysis probe in the bile duct

The bile duct microdialysis probes were constructed in-house (Figure 1). Silica tubing (40 μm i.d., 140 μm o.d.; SGE, Australia) was connected to both ends of a 7-cm dialysis membrane which was then inserted into a length of polyethylene tubing (PE-60; 0·76 mm i.d., 1·22 mm o.d.; Clay Adams, MD). PE-10 tubing (0·28 mm i.d., 0·61 mm o.d.; Clay Adams) was also connected to both ends of the PE-60 tubing and all unions were cemented with epoxy resin (Scott & Lunte 1993; Hadwiger et al 1994). After bile duct cannulation, and with the body of the anaesthetized rat maintained at 37°C, the probe was then perfused with Ringer's solution. After dialysate levels had stabilized (approx. 2 h), drug-free samples were collected and chloramphenicol succinate (100 mg kg^{-1}) was then administered intravenously via the femoral vein. Each dialysate sample (10 μL) from the microdialysis probe was assayed by on-line microdialysis coupled with microbore HPLC.

Calibration of the microdialysis probe

The in-vitro bile microdialysis probes were calibrated by perfusion with Ringer's solution with the probes containing 500 or 1000 ng mL^{-1} chloramphenicol and chloramphenicol glucuronide. The perfusion media and pumping flow-rates were both 1 μL min^{-1}. In-vitro probe recoveries were calculated by dividing the concentration in the dialysate (C_{out}) by the nominal concentration in the bile to which drug had been added (C_{in}), i.e. in-vitro recovery = C_{out}/C_{in}.

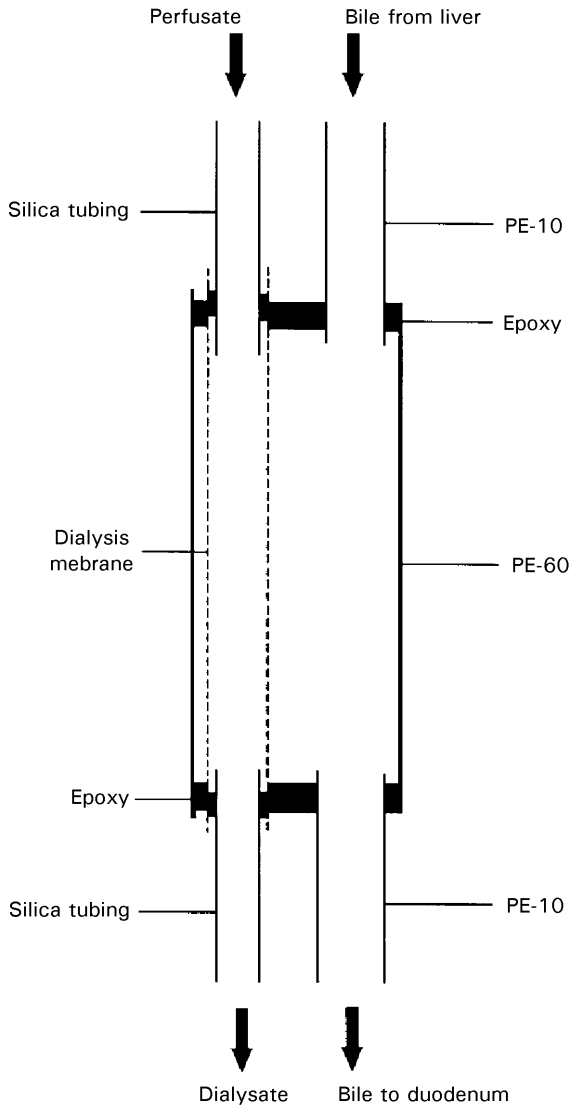

Figure 1. Flow-through microdialysis probe used for sampling rat bile.

All calibration curves were required to have a correlation coefficient of at least 0·995. The intra-day and inter-day variabilities were determined by quantifying six replicates at concentrations of 20, 50, 100, 200 and 500 ng mL^{-1} using the HPLC method described above on the same day and on four different days, respectively. The accuracy was calculated from the nominal concentration (C_{nom}) and the mean value of the observed concentrations (C_{obs}), i.e. accuracy (%) = [($C_{nom}-C_{obs}$)/C_{nom}] × 100. The precision, determined as the coefficient of variation (CV), was calculated from the observed concentrations, i.e. precision (%) = [standard deviation (s.d.)/C_{obs}] × 100. The same data were used to determine both accuracy and precision (Tsai et al 1998).

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated from individual concentration data corrected for in-vitro recovery. Unbound chloramphenicol and chloramphenicol glucuronide data were fitted to biexponential and monoexponential decay curves, respectively, according to the formulae $C = Ae^{-\alpha t} + Be^{-\beta t}$ and $C = Ae^{-\alpha t}$, respectively. The distribution and elimination rate constants, α and β were calculated by use of the equation α or $\beta = (\ln C_1 - \ln C_2)/(t_2 - t_1)$, where C_1 and C_2 were the concentrations at times t_1 and t_2, respectively.

Formation rate constants were calculated from the extrapolated formation slope determined by the method of residuals. The areas under the concentration–time curves (AUCs) were calculated by the trapezoidal rule and extrapolated to infinite time by addition of $AUC_{t-\infty} = C_t/\beta$. The AUC values for bile were thus given by the sum of the products of the measured concentrations and the collection time interval, plus the residual area; i.e. $AUC = AUC_{0-t} + AUC_{t-\infty}$. Half-life ($t_{1/2}$) values were calculated by use of the equation $t_{1/2\alpha} = 0{\cdot}693/\alpha$ and $t_{1/2\beta} = 0{\cdot}693/\beta$ for distribution and elimination half-life, respectively (Gibaldi & Perrier 1982).

Comparisons of chloramphenicol and chloramphenicol glucuronide data were performed by Student's one-tailed *t*-test. Statistical significance was accepted for $P < 0{\cdot}05$.

Results

The microbore liquid chromatographic system was used for simultaneous determination of chloramphenicol and its glucuronide in the rat bile duct. Under the conditions described above the HPLC retention times of chloramphenicol glucuronide and chloramphenicol were 5·3 and 8·0 min, respectively (Figure 2). Figure 2A shows the chromatogram obtained from a standard injection of chloramphenicol and its glucuronide (5 μg mL^{-1} each) and Figure 2B shows that from a bile dialysate sample containing chloramphenicol (6·11 μg mL^{-1}) and chloramphenicol glucuronide (76·12 μg mL^{-1}) obtained by bile duct microdialysis 60 min after intravenous administration of chloramphenicol succinate (100 mg kg^{-1}).

Table 1 In-vitro recoveries of chloramphenicol and chloramphenicol glucuronide by the bile microdialysis probe.

Concentration (ng mL^{-1})	Recovery (%)	
	Chloramphenicol	Chloramphenicol glucuronide
1000	50·49 ± 4·29	47·56 ± 1·68
500	55·32 ± 3·22	51·08 ± 3·68

Data are expressed as means ± s.e.m. (n = 6).

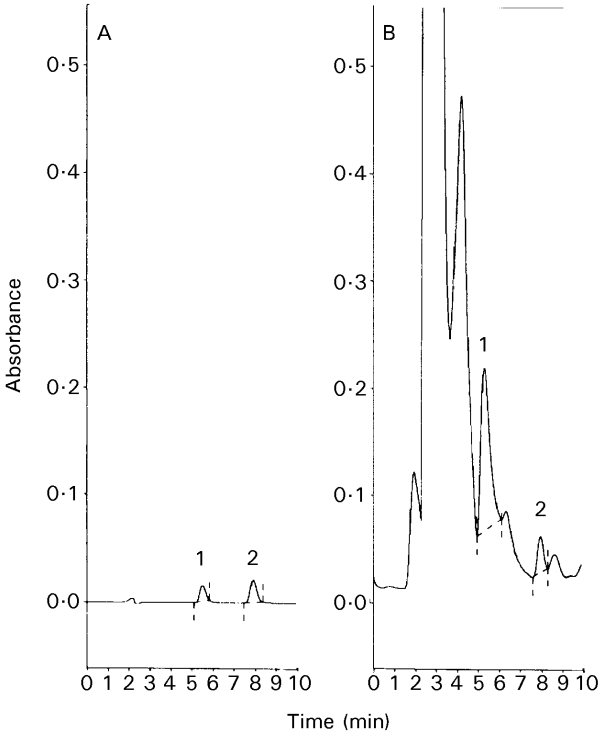

Figure 2. Typical chromatograms obtained from injection of A. standard chloramphenicol glucuronide (1) and chloramphenicol (2), both at 5 μg mL^{-1}, and B. a bile dialysate sample containing chloramphenicol glucuronide (76·12 μg mL^{-1}) and chloramphenicol (6·11 μg mL^{-1}) collected from the bile duct 60 min after intravenous administration of chloramphenicol succinate (100 mg kg^{-1}).

The in-vitro recoveries of chloramphenicol and chloramphenicol glucuronide at concentrations of 500 and 1000 ng mL^{-1} were not significantly different. The average recovery ranges between 47 and 55% (Table 1).

The plots of concentration against time are shown in Figure 3; the pharmacokinetic results (Table 2) reflect the apparent disposition of chloramphenicol according to a two-compartment model whereas chloramphenicol glucuronide fits a one-compartment model after administration of chloramphenicol succinate (100 mg kg^{-1}, i.v.). The clearance (CL) and AUC of chloramphenicol glucuronide were (approx.) half and twice, respectively, those of chloramphenicol.

Table 2 Pharmacokinetic parameters of chloramphenicol and its metabolite in rat bile after intravenous administration of chloramphenicol succinate (100mg kg^{-1}).

Parameter	Chloramphenicol	Chloramphenicol glucuronide
$t_{1/2\alpha}$ (min)	22·2 ± 7·6	–
$t_{1/2\beta}$ (min)	141·5 ± 19·4	311·4 ± 162·1
k_α (min^{-1})	0·04 ± 0·008	–
k_β (min^{-1})	0·0053 ± 0·00066	0·038 ± 0·0017
AUC (min mg mL^{-1})	10·1 ± 0·4	22·5 ± 0·5*
CL (L min^{-1} kg^{-1})	9·9 ± 0·4	4·4 ± 0·09*
AUMC (min^2 mg mL^{-1})	360 ± 19	1955 ± 108*

$t_{1/2\alpha}$ and $t_{1/2\beta}$ are the distribution and elimination half-lives, respectively; k_α and k_β are the distribution and elimination rate constants, respectively; AUC is the area under the concentration–time curve; CL is the clearance; and AUMC is the area under the moment curve. Data are expressed as mean ± s.e.m. (n = 6). *$P < 0.05$ compared with chloramphenicol.

Discussion

The metabolism of chloramphenicol has been studied in various species. In man, rabbit and guinea pig the major route of excretion of chloramphenicol, mostly in the form of its glucuronide, is by the kidneys, but in rat it is by way of the bile, the form excreted again being mostly the glucuronide (Uesugi et al 1974a).

Chloramphenicol is well known to be conjugated to glucuronic acid by the liver. According to the establihed metabolic scheme for chloramphenicol (Glazko et al 1949, 1950, 1952), this metabolite is excreted in the bile and readily hydrolysed by gut microflora β-glucuronidase, resulting in free chloramphenicol. It has been suggested that in rats chloramphenicol is glucuronidated in the liver and then actively excreted in the bile. Uesugi et al (1974a) have further shown that the glucuronide conjugation process for chloramphenicol is the rate-limiting step in the biliary excretion of the drug in rats. In addition, chloramphenicol returns to the blood by recirculation within the enterohepatic cycle.

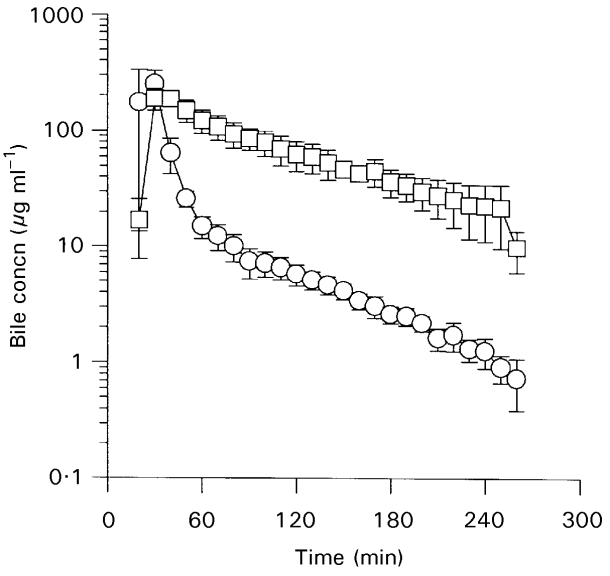

Figure 3. Plots of concentration of chloramphenicol (○) and chloramphenicol glucuronide (□) in bile against time after intravenous administration of chloramphenicol succinate (100 mg kg^{-1}) in the rat.

In our method, microdialysis reflects the free unbound drug concentration in the biological fluid or tissue. Although most research papers do not report the concentration of the free drug, it is usually the active free drug fraction, which is not bound to proteins or other macromolecules, that is most important to drug efficacy and toxicity. The current experiments thus extend our knowledge of the biliary excretion of unbound chloramphenicol and its glucuronide in rat. Over the time-course of the study, biliary excretion of unbound chloramphenicol glucuronide was approximately twice that of chloramphenicol. These results are consistent with those reported by Uesugi et al (1974b), who measured the protein-bound forms of chloramphenicol and chloramphenicol glucuronide.

Improved analytical methods have been developed for the determination of specific metabolites of chloramphenicol (Wal et al 1980), and extensive discussion of the analytical methods used for biological samples can be found elsewhere (Bories et al 1983; Cravedi et al 1994). However, it is not always convenient to apply such complicated extraction and analysis processes to biological samples. In contrast, this paper describes a straightforward microdialysis technique coupled with automatic, and sensitive, on-line microbore high-performance liquid chromatography which can easily be used for the determination of chlor-

amphenicol and chloramphenicol glucuronide in rat bile dialysates.

Apart from its simplicity and ease of use, on-line flow-through microdialysis also avoids loss of bile fluid and minimizes physiological disturbance of the animal (Scott & Lunte 1993; Hadwiger et al 1994). This, in turn, enables biliary excretion to be studied under conditions that more closely approximate normality than is possible with some other techniques used to collect bile fluid (Uesugi et al 1974b; Kates & Tozer 1976). Moreover, this technique provides protein-free samples amenable to direct injection into a chromatographic system for analysis (Johansen et al 1997).

In this study we also found that the clearance of chloramphenicol glucuronide by bile excretion was significantly more rapid than that of chloramphenicol; the ratio of the AUCs of chloramphenicol glucuronide and chloramphenicol was approximately 2 : 1 (Table 2). The conjugating enzyme in rat liver presumably explains why so much of the chloramphenicol exists in its conjugated glucuronide form (Uesugi et al 1974a).

Acknowledgements

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