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On-line microdialysis coupled with microbore liquid chromatography for the determination of unbound chloramphenicol and its glucuronide in rat blood

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Abstract

On-line microdialysis coupled with microbore liquid chromatography was used to investigate the pharmacokinetics of chloramphenicol and its glucuronide in rat blood. A microdialysis probe was inserted into a jugular vein of male Sprague–Dawley rats. Chloramphenicol succinate (20 mg/kg, intravenously) was then administered via a femoral vein. Dialysates were automatically injected onto a LC system, via an on-line injector. Samples were eluted with a mobile phase containing acetonitrile-10 m*M* monochloroacetic acid (30:70, v/v, pH 3.0). The UV detector wavelength was set at 278 nm. The limit of quantitation for chloramphenicol was 10 ng/ml. The in vitro recoveries of chloramphenicol and chloramphenicol glucuronide at 500 ng/ml were 32.2±0.3% and 11.4±0.7%, respectively (n=6). Intra- and inter-assay accuracy and precision of the analyses were ≤10% in the range of 0.01 to 5.0 μg/ml.

Keywords: Chloramphenicol

1. Introduction

Chloramphenicol (Fig. 1) has a broad spectrum of antibacterial activity. It inhibits bacterial protein synthesis by blocking the transfer of soluble ribonucleic acid to ribosome. Chloramphenicol succinate, an inactive form, may be transformed into free chloramphenicol, an active form, by hydrolysis in the liver [1].

Many LC–UV [2–5] and GC [6–8] methods have already been described for determination of chloramphenicol in different tissues or biological fluids. Microdialysis is a very powerful technique in pharmacokinetic studies. We describe here an on-line microdialysis coupled with a sensitive microbore LC–UV assay for the simultaneous determination of unbound chloramphenicol and its glucuronide in dialysate samples taken from a rat's jugular vein. Drug concentrations in microdialysates reflect the free, unbound drug and its metabolite(s) from the

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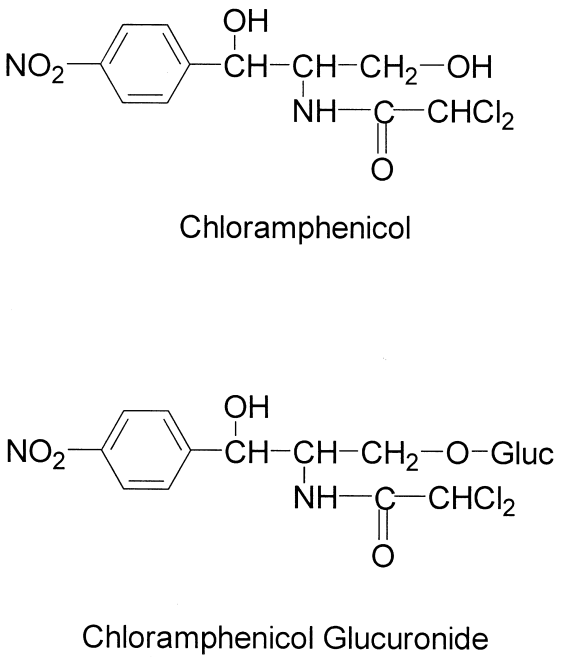

Fig. 1. Chemical structures of chloramphenicol and chloramphenicol glucuronide.

extracellular space of most tissues [9–14]. These unbound concentrations are pharmacologically active and important to drug efficacy and toxicity. In addition, microdialysis sampling technique provides an advantage of clean-up procedure prior to LC analysis [15–17].

2. Experimental

2.1. Reagents

Chloramphenicol, (*o*-[-]-threo-2-dichloroacetamido-1-[*p*-nitrophenyl]1,3-propanediol),chloramphenicol glucuronide and chloramphenicol succinate sodium salt were purchased from Sigma (St. Louis, MO, USA). The monochloroacetic acid and reagents were obtained from Merck (Darmstadt, Germany). Triple de-ionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2. Liquid chromatography

The liquid chromatographic system consisted of a chromatographic pump (BAS PM-80, West Lafayette, IN, USA), an on-line injector (CMA/160, Stockholm, Sweden) equipped with a 10 μl sample loop and a micro UV detector (Soma S-3702 ultraviolet detector, Tokyo, Japan). Analytes were separated using a reversed phase microbore column (BAS, RP-18, 150×1 mm I.D.; 5 μm). Chromatography was performed at ambient temperature. The mobile phase consisted of acetonitrile–10 m*M* monochloroacetic acid (pH 3) (30:70, v/v) with flow rate 0.05 ml/min. The mobile phase mixture was filtered through a 0.22 μm Millipore membrane. The UV wavelength was set at 278 nm. Output data from the detector were integrated via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.3. Animals

Adult, male Sprague–Dawley rats (280–320 g) were obtained from the Laboratory Animal Center at National Yang-Ming University (Taipei, Taiwan). These animals were pathogen free and kept in environmentally controlled quarters (24±1°C and 12–12 h light–dark cycle). The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the rat's body temperature was maintained at 37°C with a heating blanket.

2.4. Microdialysis experiments

The blood microdialysis system consisted of a CMA/100 microinjection pump and a CMA/160 on-line microdialysis injector. Blood dialysis probes were made of silica capillary and were of concentric designed dialysis membrane (Spectrum, 10 mm length, 150 μm outer diameter with a cut-off at nominal molecular weight of 13 000, Laguna Hills, CA, USA). The microdialysis probe was inserted into the right jugular vein and perfused with ACD solution (citric acid 3.5 m*M*; sodium citrate 7.5 m*M*; dextrose 13.6 m*M*) at a flow-rate of 1 μl/min using the CMA/100 microinjection pump [16]. After obtaining 2 h base-line collection, chloramphenicol

succinate (20 mg/kg) was intravenously administered via a femoral vein. Dialysis samples were collected every 10 min and 10 μl of dialysate was assayed with microbore LC/UV.

2.5. In vitro recovery

The blood microdialysis probe was calibrated by inserting it into a sample vial containing 500 ng/ml of chloramphenicol and chloramphenicol glucuronide in ACD solution. The perfusion media and pumping flow-rate were the same as described above. The probe recovery was calculated by dividing the concentrations in the dialysate (C_{out}) by the concentration in the tube (C_{in}) [18], that is: recovery $_{in\ vitro} = C_{out}/C_{in}$.

2.6. Method validation

All calibration curves of analytes were made prior to on-line microdialysis experiments with correlation values of at least 0.995. The intra-assay and inter-assay variabilities for chloramphenicol and chloramphenicol glucuronide in ACD solution were assayed ($n=6$) at concentrations of 0.05, 0.1, 1, and 5 μg/ml on the same day and on four different days, respectively. These same data were also used to calculate the accuracy between the nominal (C_{nom}) and observed concentration (C_{obs}). Accuracy was expressed

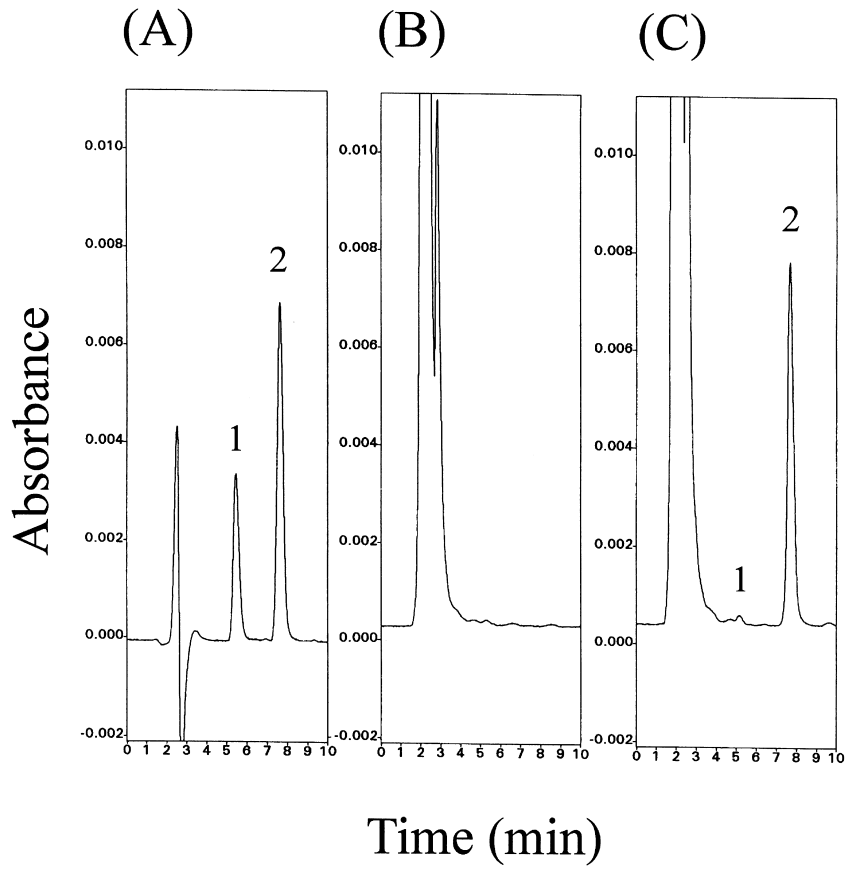

Fig. 2. Typical chromatogram of injection of (A) standard chloramphenicol glucuronide (1) and chloramphenicol (2; both at 0.5 μg/ml), (B) a blank blood dialysate, and (C) a blood dialysate sample containing chloramphenicol glucuronide (0.13 μg/ml) and chloramphenicol (0.62 μg/ml) collected from jugular vein at 60 min after chloramphenicol succinate administration (20 mg/kg, i.v.).

as a percentage bias as follows: Bias (%)=100× (C_{nom}–C_{obs})/ C_{nom}. Accuracy (% bias) and precision (% CV) values of within ±20% were considered acceptable over this concentration range [19].

3. Results and discussion

3.1. Specificity of chloramphenicol and its glucuronide in blood microdialysate

The present microbore liquid chromatographic method was applied to simultaneously determine chloramphenicol and its glucuronide from rat jugular vein. Under the condition described above, the retention times of chloramphenicol glucuronide and chloramphenicol were found to be 5.7 and 7.4 min, respectively (Fig. 2). Fig. 2A shows a standard injection of chloramphenicol and its glucuronide (0.5 μg/ml each). Fig. 2B shows a chromatogram of a blank blood dialysate. No peaks were observed that would interfere with the analysis of either compound. Fig. 2C shows a chromatogram of a blood dialysate sample containing chloramphenicol (0.62 μg/ml) and chloramphenicol glucuronide (0.13 μg/ml) obtained from blood microdialysis 60 min after chloramphenicol succinate administration (20 mg/kg, i.v.).

3.2. Linearity, precision and accuracy

The method was linear (r^2>0.995) over a concentration range 0.01–5 μg/ml for both chloramphenicol and chloramphenicol glucuronide. Intra-day and inter-day precision and accuracy for both chloramphenicol (Table 1) and chloramphenicol glucuronide (Table 2) fell well within predefined limits of acceptability. All % bias and % CV values were within ±15%.

3.3. Microdialysis

The in vitro recoveries of chloramphenicol and chloramphenicol glucuronide were 32.2±0.3% and 11.4±0.7%, respectively, (based on 500 ng/ml standards mixture). The concentrations of chloramphenicol and its glucuronide in dialysates of rat blood after chloramphenicol succinate (20 mg/kg, i.v.) administration are shown in Fig. 3. The samples were collected at 10 min intervals during the entire experimental period. As described above, this method is applicable to further pharmacokinetic studies on chloramphenicol and its glucuronide.

Table 1
Intra- and inter-assays variabilities for chloramphenicol in rat plasma

Nominal concentration (μg/ml)	Observed concentration (μg/ml)[a]	CV (%)	Accuracy (% bias)
Intra-assay (n=6)			
0.05	0.051±0.0041	8.1	1.6
0.1	0.10±0.0041	4.0	2
0.5	0.50±0.0089	1.8	0.1
1	0.98±0.018	1.8	−1.8
5	5.01±0.0052	0.1	0.06
Inter-assay (n=4)			
0.05	0.052±0.0045	8.7	3
0.1	0.10±0.0079	7.9	0.3
0.5	0.51±0.019	3.8	1
1	0.99±0.026	2.6	−0.8
5	4.99±0.0045	0.1	−0.04

[a] Observed concentration data are expressed as rounded means±S.D.

Table 2
Intra- and inter-assays variabilities for chloramphenicol glucuronide in rat plasma

Nominal concentration (μg/ml)	Observed concentration (μg/ml)a	CV (%)	Accuracy (% bias)
Intra-assay (n=6)			
0.05	0.051±0.0022	4.3	0.6
0.1	0.098±0.004	4.1	−2
0.5	0.502±0.021	4.2	0.4
1	1.01±0.016	1.6	1.3
5	5.01±0.0052	0.3	0.2
Inter-assay (n=4)			
0.05	0.043±0.0038	8.8	−14
0.1	0.102±0.0066	6.5	2
0.5	0.502±0.0041	0.8	0.4
1	0.995±0.027	2.7	−0.5
5	4.996±0.0055	0.1	−0.08

[a] Observed concentration data are expressed as rounded means±S.D.

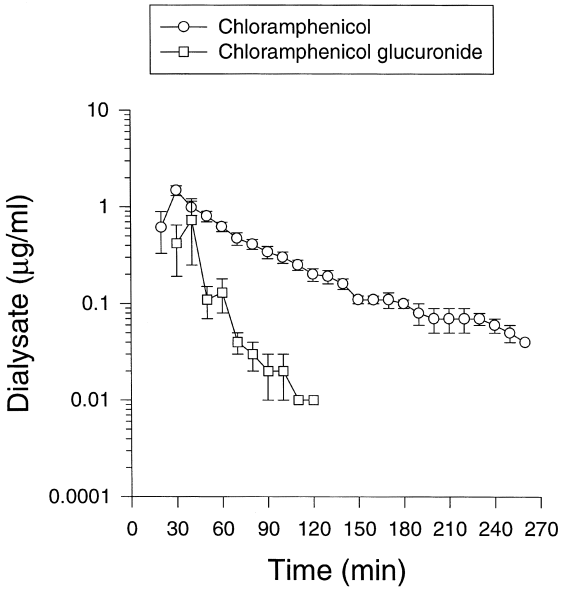

Fig. 3. Mean dialysate levels of chloramphenicol (○) and its glucuronide (□) in blood of the jugular vein after chloramphenicol succinate administration (20 mg/kg, i.v.).

4. Conclusion

This microdialysis technique provides protein-free samples that can be directly injected onto a liquid chromatographic system for continuous in vivo monitoring of unbound drugs in blood. Further, this sampling method facilitates pharmacokinetic studies with reducing the effects of blood volume changes as compared to conventional blood withdrawing assay. Its potential for studying the pharmacokinetics of chloramphenicol in rat blood was demonstrated here.

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References

[1] M.C. Nahata, D.A. Powell, Clin Pharmacol. Ther. 30 (1981) 368.
[2] E.H. Allen, J. Assoc. Off. Anal. Chem. 68 (1985) 990.
[3] C. Hummert, B. Luckas, H. Siebenlist, J. Chromatogr. B 668 (1995) 53.
[4] D.E. Holt, R. Hurley, D. Harvey, J. Antimicrob. Chemother. 35 (1995) 115.
[5] B.A. De Craene, P. Deprez, E. D'haese, H.J. Nelis, W. Van den Bossche, A.P. De Leenheer, Antimicrob. Agents Chemother. 41 (1997) 1991.
[6] T. Gude, A. Preiss, K. Rubach, J. Chromatogr. B 673 (1995) 197.
[7] L.A. van Ginkel, H.J. van Rossum, P.W. Zoontjes, H. van Blitterswijk, G. Ellen, E. van der Heeft, A.P.J.M. de Jong, G. Zomer, Anal. Chim. Acta 237 (1990) 61.
[8] D. Arnold, A. Somogyi, J. Assoc. Off. Anal. Chem. 68 (1985) 984.
[9] J. Bolinder, E. Hagstrom, U. Ungerstedt, P. Arner, Scand., J. Clin. Lab. Invest. 49 (1989) 465.
[10] L. Stahle, U. Ungerstedt, Psychopharmacology 99 (1989) 75.
[11] M. Telting-Diaz, D.O. Scott, C.E. Lunte, Anal. Chem. 64 (1992) 806.
[12] K.M. Steele, C.E. Lunte, J. Pharm. Biomed. Anal. 13 (1995) 149.
[13] J.A. Stenken, C.E. Lunte, M.Z. Southard, L. Stahle, J. Pharm. Sci. 86 (1997) 958.
[14] T.H. Tsai, C.F. Chen, J. Chromatogr. A 730 (1996) 121.
[15] U. Ungerstedt, in: C.A. Arsden (Ed.), Measurement of Neurotransmitter Release in Vivo, John Wiley & Sons, New York, 1984, p. 81.
[16] T.H. Tsai, C.F. Chen, Neurosci. Lett. 166 (1994) 175.
[17] M.J. Johansen, R.A. Newman, T. Madden, Pharmacotherapy 17 (1997) 464.
[18] H. Benveniste, A.J. Hansen, in: T.E. Robinson, J.B. Justice Jr. (Eds.), Microdialysis in the Neurosciences, Elsevier, Amsterdam, 1991, p. 81.
[19] R. Causon, J. Chromatogr. B 689 (1997) 175.