

Supporting Information

Blood-placental barrier transfers and pharmacokinetics of unbound morphine in pregnant rats with multiple microdialysis systems

I-Hsin Lin¹, Ling Yang¹, Thomas Y. Hsueh^{2,3}, and Tung-Hu Tsai^{1,4,5*}

¹ Institute of Traditional Medicine, School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan

² Division of Urology, Department of Surgery, Taipei City Hospital Renai Branch, Taipei, Taiwan

³ Department of Urology, School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan

⁴ Graduate Institute of Acupuncture Science, China Medical University, Taichung, Taiwan

⁵ Department of Psychology, University of Cambridge, Cambridge CB2 3EB, UK

*Author for correspondence:

Tung-Hu Tsai, PhD, Professor

Institute of Traditional Medicine, School of Medicine, National Yang Ming Chiao Tung University, 155, Li-Nong Street Section 2, Taipei 112, Taiwan

Fax: (886-2) 2822 5044; Tel: (886-2) 2826 7115; E-mail: thtsai@nycu.edu.tw

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Table S1. Intra- and inter-day precision (% RSD) and accuracy (% Bias) of the UHPLC-MS/MS method for the determination of morphine in rat maternal blood, placenta, fetus, and amniotic fluid dialysates.

Nominal concentration (ng/mL)	Intra-day			Inter-day		
	Observed concentration (ng/mL)	Precision (% RSD)	Accuracy (% Bias)	Observed concentration (ng/mL)	Precision (% RSD)	Accuracy (% Bias)
Maternal blood						
1	0.89 ± 0.04	4.84	-11.11	1.06 ± 0.17	16.10	5.78
5	4.81 ± 0.08	1.58	-3.80	4.82 ± 0.57	11.78	-3.67
100	101.5 ± 0.40	0.40	1.57	101.5 ± 3.82	3.77	1.52
500	498.6 ± 3.53	0.71	0.71	499.8 ± 0.56	0.11	-0.04
Placenta						
1	1.16 ± 0.13	10.90	16.45	1.03 ± 0.16	15.02	3.36
5	5.10 ± 0.12	2.44	2.04	4.84 ± 0.30	6.10	-3.16
100	94.06 ± 1.08	1.15	-5.94	101.0 ± 3.11	3.08	1.03
500	448.9 ± 31.63	7.05	-10.21	499.9 ± 0.49	0.10	-0.02
Fetus						
1	1.20 ± 0.22	18.07	19.90	0.88 ± 0.08	9.52	-11.72
5	4.97 ± 0.16	3.23	-0.61	4.87 ± 0.37	7.50	-2.62
100	94.89 ± 1.59	1.67	-5.11	101.5 ± 4.04	3.98	1.51
500	456.5 ± 24.80	5.43	-8.69	499.8 ± 0.48	0.10	-0.04
Amniotic fluid						
1	0.98 ± 0.18	18.81	-1.96	1.18 ± 0.16	13.36	17.53
5	5.02 ± 0.21	4.20	0.36	4.93 ± 0.34	6.90	-1.41
100	108.7 ± 2.72	2.51	8.67	104.6 ± 1.99	1.90	4.61
500	502.6 ± 5.61	1.12	0.53	499.9 ± 0.26	0.05	-0.03

Data are expressed as Mean ± SD (n = 5).

Table S2. Intra- and inter-day precision (% RSD) and accuracy (% Bias) of the UHPLC-MS/MS method for the determination of M3G in rat maternal blood, placenta, fetus, and amniotic fluid dialysates.

Nominal concentration (ng/mL)	Intra-day			Inter-day		
	Observed concentration (ng/mL)	Precision (% RSD)	Accuracy (% Bias)	Observed concentration (ng/mL)	Precision (% RSD)	Accuracy (% Bias)
Maternal blood						
1	0.99 ± 0.03	3.26	-1.25	1.13 ± 0.20	17.56	13.06
5	4.96 ± 0.03	0.51	-0.71	5.14 ± 0.38	7.48	2.80
100	101.9 ± 2.21	2.17	1.94	99.17 ± 3.18	3.21	-0.83
500	498.2 ± 23.58	4.73	-0.35	500.2 ± 0.46	0.09	0.03
Placenta						
1	0.97 ± 0.10	9.90	-2.93	0.99 ± 0.20	19.85	-1.03
5	5.26 ± 0.16	3.09	5.31	4.90 ± 0.31	6.41	-2.08
100	100.3 ± 0.29	0.29	0.39	99.30 ± 3.37	3.39	-0.70
500	517.5 ± 10.27	1.99	-3.49	500.2 ± 0.50	0.10	0.04
Fetus						
1	1.05 ± 0.07	6.72	4.87	1.02 ± 0.19	18.76	2.00
5	5.73 ± 0.12	2.18	14.60	5.07 ± 0.37	7.23	1.49
100	98.05 ± 2.58	2.64	-1.95	99.06 ± 4.32	4.36	-0.94
500	481.6 ± 11.03	2.29	-3.68	500.0 ± 0.25	0.05	-0.01
Amniotic fluid						
1	1.19 ± 0.04	3.22	18.84	1.19 ± 0.22	18.34	18.78
5	5.29 ± 0.18	3.32	5.73	5.12 ± 0.24	4.63	2.34
100	98.04 ± 14.11	14.39	-1.96	98.89 ± 3.09	3.13	-1.11
500	478.2 ± 13.49	2.82	-4.36	500.3 ± 0.42	0.08	0.06

Data are expressed as Mean ± SD (n = 5).

Table S3. Intra- and inter-day precision (% RSD) and accuracy (% Bias) of the UHPLC-MS/MS method for the determination of M6G in rat maternal blood, placenta, fetus, and amniotic fluid dialysates.

Nominal concentration (ng/mL)	Intra-day			Inter-day		
	Observed concentration (ng/mL)	Precision (% RSD)	Accuracy (% Bias)	Observed concentration (ng/mL)	Precision (% RSD)	Accuracy (% Bias)
Maternal blood						
5	4.82 ± 0.25	5.26	-3.60	5.17 ± 0.39	7.50	3.36
10	9.80 ± 0.25	2.58	-1.91	9.95 ± 1.21	12.20	-0.48
500	499.1 ± 3.66	0.73	-0.17	508.5 ± 28.57	5.62	1.69
1000	1000 ± 14.83	1.48	0.03	1000 ± 3.17	0.32	0.03
Placenta						
5	4.97 ± 0.24	4.74	-0.51	5.92 ± 1.12	18.92	18.37
10	10.00 ± 0.27	2.72	0.02	11.06 ± 1.20	10.88	10.65
500	489.3 ± 9.45	1.93	-2.14	500.8 ± 4.07	0.81	0.15
1000	986.9 ± 26.73	2.71	-1.31	1000 ± 2.00	0.20	-0.02
Fetus						
5	4.86 ± 0.22	4.62	-2.91	5.31 ± 0.83	15.67	6.28
10	9.69 ± 0.17	1.80	-3.06	11.07 ± 1.12	10.09	10.71
500	485.9 ± 8.15	1.68	-2.81	495.2 ± 7.18	1.45	-0.96
1000	999.2 ± 6.33	0.63	-0.08	1002 ± 3.40	0.34	0.23
Amniotic fluid						
5	5.29 ± 0.22	4.15	5.81	4.90 ± 0.70	14.20	-2.03
10	9.96 ± 0.24	2.46	-0.36	10.23 ± 0.69	6.79	2.29
500	436.3 ± 36.03	8.26	-12.75	503.7 ± 8.44	1.68	0.75
1000	923.5 ± 44.05	4.77	-7.65	998.2 ± 3.72	0.37	-0.17

Data are expressed as Mean ± SD (n = 5).

Table S4. Stability of morphine in rat maternal blood, placenta, fetus, and amniotic fluid dialysates.

Nominal concentration (ng/mL)	Autosampler stability (%)	Short-term stability (%)	Freeze-thaw stability (%)	Long-term stability (%)
Maternal blood				
5	98.81 ± 2.21	89.44 ± 2.95	99.60 ± 3.10	92.87 ± 0.22
500	96.70 ± 0.56	92.84 ± 1.26	102.3 ± 0.41	87.32 ± 1.02
Placenta				
5	98.90 ± 1.13	91.93 ± 1.35	99.52 ± 2.18	91.00 ± 3.19
500	101.6 ± 0.37	91.78 ± 0.67	100.9 ± 1.62	91.35 ± 3.22
Fetus				
5	98.00 ± 1.06	90.68 ± 3.98	100.2 ± 1.77	93.24 ± 3.44
500	98.09 ± 1.25	87.97 ± 0.41	100.4 ± 0.85	89.84 ± 1.13
Amniotic fluid				
5	99.30 ± 2.08	88.82 ± 2.22	97.97 ± 0.79	94.60 ± 0.64
500	97.16 ± 0.54	88.45 ± 0.57	98.17 ± 0.98	90.56 ± 1.48

Data are expressed as Mean ± SD (n = 3).

Table S5. Stability of M3G in rat maternal blood, placenta, fetus, and amniotic fluid dialysates.

Nominal concentration (ng/mL)	Autosampler stability (%)	Short-term stability (%)	Freeze-thaw stability (%)	Long-term stability (%)
Maternal blood				
5	104.2 ± 1.69	100.5 ± 2.14	100.3 ± 3.62	101.0 ± 4.41
500	104.2 ± 1.24	100.2 ± 0.78	105.1 ± 1.45	105.8 ± 0.74
Placenta				
5	100.2 ± 1.75	98.00 ± 4.45	103.7 ± 0.73	98.34 ± 1.34
500	100.4 ± 0.60	101.0 ± 1.92	101.5 ± 0.95	97.25 ± 9.67
Fetus				
5	99.90 ± 1.87	99.29 ± 3.05	102.6 ± 1.10	101.3 ± 0.94
500	98.50 ± 0.43	98.03 ± 1.16	103.6 ± 0.46	102.4 ± 0.40
Amniotic fluid				
5	99.86 ± 0.86	99.28 ± 4.40	100.4 ± 1.39	102.6 ± 2.87
500	98.14 ± 0.48	101.3 ± 1.35	99.30 ± 0.55	101.0 ± 6.99

Data are expressed as Mean ± SD (n = 3).

Table S6. Stability of M6G in rat maternal blood, placenta, fetus, and amniotic fluid dialysates.

Nominal concentration (ng/mL)	Autosampler stability (%)	Short-term stability (%)	Freeze-thaw stability (%)	Long-term stability (%)
Maternal blood				
10	98.93 ± 0.98	92.59 ± 1.79	99.13 ± 2.76	92.54 ± 1.63
1000	98.23 ± 0.76	93.12 ± 5.87	101.0 ± 0.82	94.73 ± 1.36
Placenta				
10	100.0 ± 1.81	90.78 ± 0.90	101.1 ± 1.94	91.77 ± 3.05
1000	100.3 ± 1.01	91.34 ± 1.18	99.59 ± 1.63	92.48 ± 7.41
Fetus				
10	99.07 ± 1.04	93.90 ± 3.16	101.8 ± 0.86	93.68 ± 0.59
1000	99.89 ± 0.12	90.91 ± 1.31	99.82 ± 1.60	96.36 ± 0.44
Amniotic fluid				
10	99.35 ± 0.56	91.50 ± 2.67	101.0 ± 2.23	92.88 ± 1.56
1000	99.55 ± 0.99	89.51 ± 2.18	97.61 ± 0.35	93.82 ± 4.56

Data are expressed as Mean ± SD (n = 3).

Table S7. *In vitro* microdialysis recovery (%) of morphine and M3G in blood and placenta probe using ACD solution as perfusion solution.

Concentration (ng/mL)	Recovery (%) in blood probe	Recovery (%) in placenta probe
Morphine		
100	25.86 ± 2.83	13.91 ± 0.91
500	24.90 ± 1.95	14.15 ± 0.88
1000	25.33 ± 0.73	14.87 ± 1.36
average	25.36 ± 0.48	14.31 ± 0.50
M3G		
100	7.09 ± 0.39	3.92 ± 0.20
500	7.44 ± 0.39	4.26 ± 0.49
1000	6.96 ± 0.60	4.04 ± 0.22
average	7.16 ± 0.25	4.07 ± 0.17

Data are expressed as Mean ± SD (n = 3).

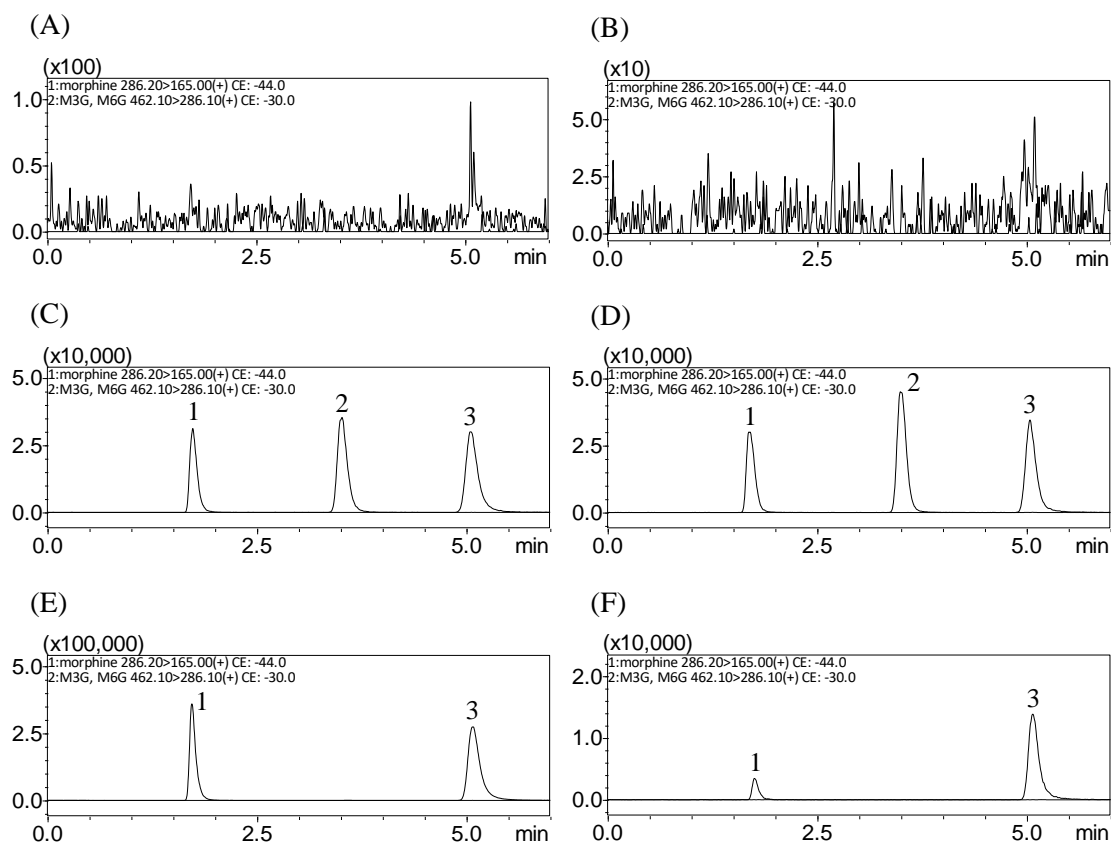


Figure S1. Representative MRM chromatograms of (A) blank maternal blood dialysate, (B) blank placenta dialysate, (C) blank maternal blood dialysate spiked with M3G (100 ng/mL), morphine (100 ng/mL), and M6G (500 ng/mL), (D) blank placenta dialysate spiked with M3G (100 ng/mL), morphine (100 ng/mL), and M6G (500 ng/mL), (E) maternal blood dialysate sample containing M3G (978.01 ng/mL) and morphine (938.61 ng/mL) collected at 40 min after morphine administration (10 mg/kg, i.v.), (F) placenta dialysate sample containing M3G (12.58 ng/mL) and morphine (42.14 ng/mL) collected at 100 min after morphine administration (10 mg/kg, i.v.); peak 1 = M3G, peak 2 = M6G, peak 3 = morphine.

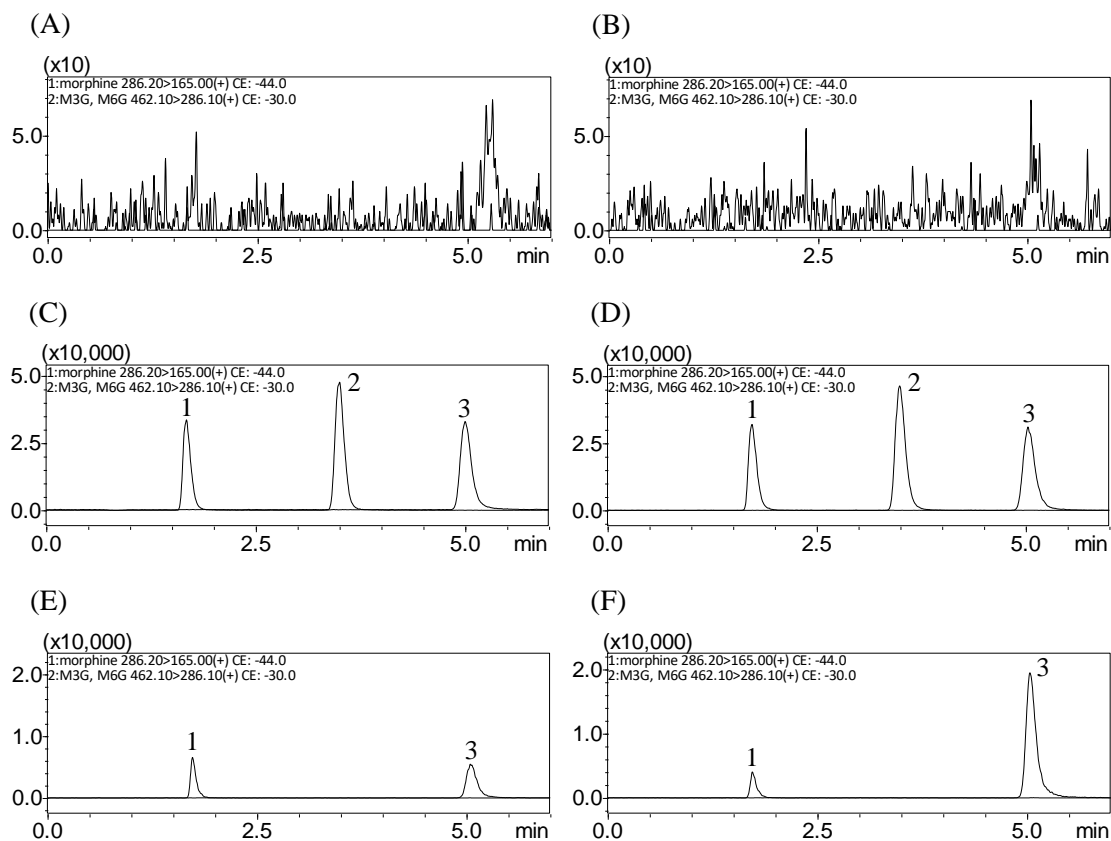


Figure S2. Representative MRM chromatograms of (A) blank fetus dialysate, (B) blank amniotic fluid dialysate, (C) blank fetus dialysate spiked with M3G (100 ng/mL), morphine (100 ng/mL), and M6G (500 ng/mL), (D) blank amniotic fluid dialysate spiked with M3G (100 ng/mL), morphine (100 ng/mL), and M6G (500 ng/mL), (E) fetus dialysate sample containing M3G (18.87 ng/mL) and morphine (16.85 ng/mL) collected at 100 min after morphine administration (10 mg/kg, i.v.), (F) amniotic fluid dialysate sample containing M3G (13.95 ng/mL) and morphine (57.67 ng/mL) collected at 100 min after morphine administration (10 mg/kg, i.v.); peak 1 = M3G, peak 2 = M6G, peak 3 = morphine.