Automated Blood Sampling: does it contribute to the 3R's?

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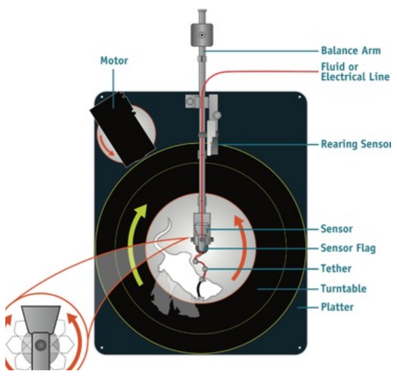


Acute blood sampling techniques such as facial vein and tail vein are commonly used techniques to obtain blood samples from mice and rats. These acute techniques require the animal to be restrained; physically (immobilization) or chemically (anesthesia). Restraint compromises the "true" value of blood drug concentration. Freely moving blood sampling - indwelling catheters - is the preferred method to obtain these "true" values (1). Automated blood sampling (ABS) in combination with freely moving animals can further contribute for obtaining "true" values of blood drug concentration. ABS significantly contributes not only to the "true" blood drug value but moreover contributes to the reduction of animals needed in stress prone research (5). Park at al. were able to investigate the difference (significant) in stress responds between wild type- and pendrin knock out mice.

Automated Blood Sampling Setup

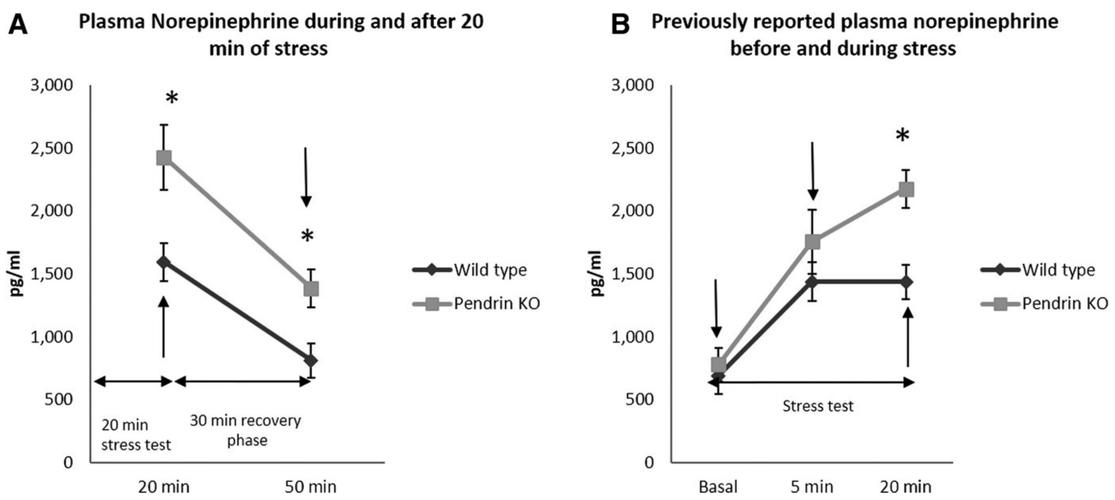
The Culex automated blood sampling setup of BASi Research Products uses an automated drug infusion and sampling device in combination with a turning cage (5). The absence of swivels enables multiple catheters, fluids samplings (blood, bile, CSF, dialysate) and sensing/stimulation (electrical and optical). The system is used in several research fields among which are safety pharmacology, PK/bioavailability, ADME and PK/PD (5).

Blood Sampling Methodology Influences Stress Hormones

The endogenous catecholamines concentration is dependent on the blood sampling method (3). This compromises the "true value" of catecholamines. Blood sampling from freely moving animals is preferred in stress research (1). Park at al. were able to investigate the difference in stress responds between wild type- and pendrin knock out mice. Additionally, from other studies (2) it is well know that handling impacts stress and metabolic endpoints such as glucose concentration. For example, most glucose clamp studies are performed in freely moving models (5).

Sampling method	Anesthetic	Age/sex	NE	E	NE+E	Reference
Decapitation	ND	20/m,f	–	–	46.1±4.8	[12]
Decapitation	ND	ND/ND	59.7±7.1	79.7±6.6		[17]
Cardiac puncture	Ether	2/ND	10.3±1.4	3.1±0.7		[2]
Cardiac puncture	Asphyxia	16–32/ND	124.1±20			[14]
Cardiac puncture	Tribromoethanol	ND/ND	21.5±2.8			[5]
Tail vein	ND	12/ND	13±1.4	0.9±0.1		[11]
Retro-orbital	ND	12/m	17.7	21.8		[10]
Retro-orbital	ND	12–32/ND	13.5±0.7	13.4±0.8		[9]
Retro-orbital	Pentobarbital	9–14/ND	1.4±0.6	1.36±0.1		[3]
Retro-orbital	Pentobarbital	10/m	6.6±1.4	0.5±0.1		[15]
Carotid catheter	ND	16–32/ND			4.7±0.8	[13]
Carotid catheter	Tribromoethanol	ND	3.8±0.6			[7]
Decapitation	No	12–18/m,f	24.6±2.7	27.3±3.8		Present study
Retro-orbital	Halothane	12–18/m,f	5.8±0.8	0.4±0.1		Present study
Carotid catheter	No	12–18/m,f	4.1±0.5	1.1±0.3		Present study

(3) Blood sampling methodology is crucial for precise measurement of plasma catecholamines concentrations in mice



(2) Park, A.Y. et al. Blood collection in unstressed, conscious, and freely moving mice through implantation of catheters in the jugular vein: a new simplified protocol

Blood Sampling Methodology Influences Pharmacokinetics of Drug Concentration

Apart from the reduction of stress by sampling blood from "freely moving" moving mice, the blood sampling methodology also impacts the drug concentration as indicated in table 2.
This has considerable consequences for the number of animals used for pharmacokinetic studies.

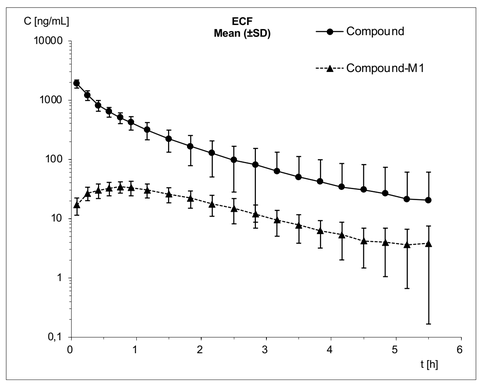
Table 1 Plasma concentrations of docetaxel (mean ± SE) at each time point obtained from cannulated and non-cannulated animals

	Cannulated	Non-cannulated	
Time (h)	Mean plasma concentration (ng/ml)	Mean plasma concentration (ng/ml)	*P*
0.08	8055.1 ± 552.6	8429.6 ± 345.8	0.57
0.25	3101.0 ± 241.0	3610.3 ± 129.9	0.08
0.5	1348.3 ± 82.6	1533.8 ± 96.5	0.21
1	578.5 ± 34.6	519.6 ± 39.3	0.29
2	227.1 ± 42.8	243.0 ± 36.0	0.78
3	124.5 ± 17.9	80.2 ± 16.3	0.55
4	74.6 ± 10.7	68.3 ± 6.1	0.60
5	63.3 ± 9.2	55.5 ± 3.1	0.61

(2) Cannulation of the jugular vein in mice: a method for serial withdrawal of blood samples

Automated Blood Sampling; Freely Moving Unattended Blood Sampling

The brain penetration of Compound was long known but not quantified. Nevertheless, the ability of its active metabolite "M1" to pass the blood brain barrier was questioned. With the present study we could demonstrate that M1 can pass the blood brain barrier, although more slowly and to a lower extent that its parent.
A direct comparison based on total plasma concentrations was not meaningful due to differences in protein-binding. Peritoneal micro-dialysis was used as surrogate for the determination of the free concentrations in plasma.



Concentration ratios of compound-M1 and compound

M1 vs. compound		
plasma	perit.	ECF
0.372	0.167	0.075
0.067	0.057	0.011
17.9	34.1	14.6

Does it contribute to the three R's?

The present study clearly demonstrates that the combination of micro-dialysis with automated blood collection in individual rats provides high quality data with a lower number of animals than to be used based on manual blood sampling. In the latter case, additional animals would be for parallel groups. In addition to obtain a full profile of plasma concentrations more than one rat would be needed.

- **Refinement**
  - o No use of restraint (acute sampling technique)
  - o Post-operative group housing
- **Reduction**
  - o Re-use of animals
  - o Data integrity (reliable results)
  - o Combination of infusion/sampling/sensing/stimulation

Literature

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