Development of sampling method for cerebrospinal fluid in mice and effect of sampling method on drug concentration in cerebrospinal fluid

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Purpose

Since the sampling of cerebrospinal fluid (CSF) from mice was difficult due to mouse body size, we developed a sampling method and a novel sampling device for the CSF from mice. We investigated the contamination ratio of blood to the CSF due to the sampling method. Furthermore, The effect of the contamination on drug concentration in the CSF was also evaluated by using tool compounds.

Materials and Methods

Development of sampling method for cerebrospinal fluid in mice : Mice and rats were anesthetized by inhalation of isoflurane, and the blood and the CSF were collected from the caudal vena cava and the cisterna magna, respectively. The weight of the collected CSF was measured. The CSF volume was estimated from the weight on the assumption of specific gravity as 1. Red blood cells in the blood and the CSF were counted. The contamination ratio of the CSF by the blood was calculated as the number ratio of red blood cells in the CSF (RBC_{CSF}) to those in the blood (RBC_{blood}).

Effect of blood contamination on drug concentration in CSF (Ccsf): Metformin, ranitidine, ibuprofen, imipramine, glimepiride, glibenclamide and nefazodone were administered to mice (8W) intraperitoneally. Plasma, brain and CSF samples were obtained after dosing. CSF was collected deliberately contaminated with blood.

All animal protocols were approved by the Institutional Animal Care and Use Committee of Shonan Research Center, Takeda Pharmaceutical Company Ltd.

Bioanalysis : Concentrations of metformin, ranitidine, ibuprofen, imipramine, glimepiride, glibenclamide and nefazodone in samples were quantified by LC/MS/MS analysis.

Unbound fraction: Unbound fraction of compounds in plasma and brain was measured by equilibrium dialysis method (fu,p and fu,b, respectively)

Results

The novel method of mouse CSF collection

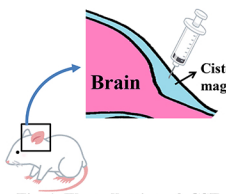

Fig. 1. The collection of CSF from mice.

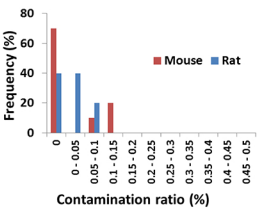

Fig. 2 Histogram of blood contamination ratio in mouse and rat CSF.

Table 1 Collection amount of mouse CSF and rat CSF

	Amount of CSF (μL)	
	Mouse	Rat
Mean	11.8	159.2
SD	2.9	26.2

$$\text{Contamination ratio} = \frac{RBC_{CSF}}{RBC_{blood}}$$

Table 2 Unbound fraction of 7 compounds in mouse plasma and brain

Compounds	fu,p	fu,b
Metformin	1.0	0.808
Ranitidine	1.0	0.571
Ibuprofen	0.12	0.263
Imipramine	0.10	0.010
Glimepiride	0.030	0.077
Glibenclamide	0.030	0.055
Nefazodone	0.020	0.004

Effect of blood contamination on Ccsf

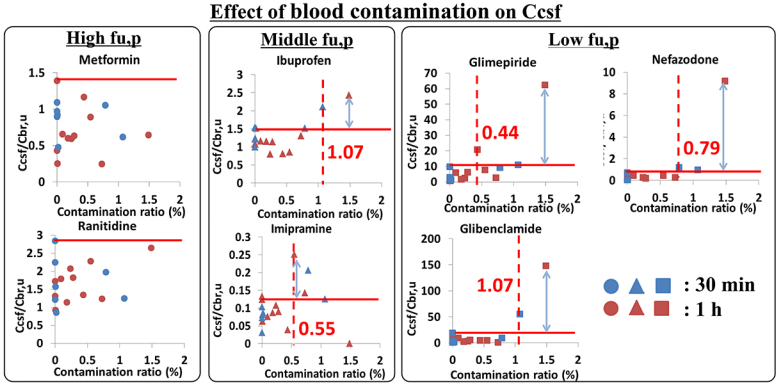

Fig. 3. Relationship of the values Ccsf /Cbr,u with contamination ratio (%).
Cbr,u = Brain conc. x fu,b. Red lines indicate maximum Ccsf/Cbr,u at which contamination is 0%.
Red dotted lines indicate minimum contamination ratio at which Ccsf/Cbr,u exceeds red line.

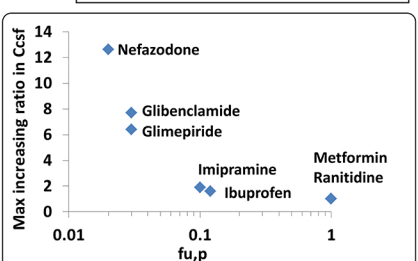

Fig. 4 Correlation between fu,p and increasing ratio by contamination in Ccsf.

Max increasing ratio in Ccsf was calculated by the following formula,
Max increasing ratio in Ccsf = [Ccsf/Cbr,u]max/[Ccsf/Cbr,u]nc.
[Ccsf/Cbr,u]max is maximum value of Ccsf/Cbr,u.
[Ccsf/Cbr,u]nc is maximum value of Ccsf/Cbr,u in samples without contamination.

Ccsf and Cbr,u by correction based on contamination ratio

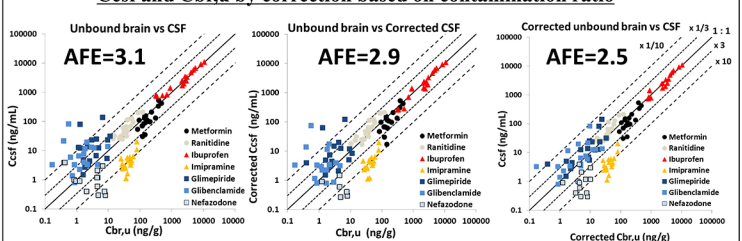

Fig. 5. The correlation between Ccsf and Cbr,u by correction based on contamination ratio.
Average-fold error (AFE) =10^{1/N x Σ|Log (Predicted/Actual)|}

$$\text{Corrected Ccsf} = \frac{\text{CSF} - \text{Plasma conc.} \times \text{Contamination ratio}}{1 - \text{Contamination ratio}} \qquad \text{Corrected Cbr,u} = \frac{\text{Cbr, u} + \text{Plasma conc.} \times \text{Contamination ratio}}{1 + \text{Contamination ratio}}$$

Summary

- ✓ We established a method of CSF sampling from mice with maximum contamination ratio of 0.14%
- ✓ Minimum contamination ratio which affected compound concentration in CSF was 0.44%.
- ✓ The compounds with lower fu,p tended to show the larger effect on concentration ratio.
- ✓ AFE value between Ccsf and Cbr,u was improved by correction based on contamination ratio.

Conclusion

We established a method of CSF sampling from mice applicable to the evaluation of compound concentration in CSF.

COI disclosure information

We have no financial relationship to disclose for our presentation contents.