OTHER

IMMUNOAFFINITY MASS SPECTROMETRY FOR LEUKOTRIENE ANALYSIS IN BRAIN

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/ CONTEXT

Leukotrienes (LTs) are inflammatory molecules which play an important role in triggering inflammatory diseases such as asthma, neurological diseases and diabetes. As a result, it is important to better understand their role and function in terms of disease progression and their potential value as biomarkers. Due to difficulty of isolation and quantification of these molecules from biological tissues, two different approaches were explored and compared in this study. In both methods, mouse brain tissue homogenates, prepared using the Precellys® Evolution, were used for isolation and quantification of LTs. The results showed that immunoaffinity (IA) enrichment method performed better and proved to be a better sample preparation method for simultaneous measurements of LTs (LTB(4), LTC(4), LTD(4) and LTE(4)) when compared to the commonly used solid phase extraction (SPE) approach.

/ MATERIALS

- Precellys® Evolution beat beating homogenizer
- Precellys® Lysing Kit: CK28_7mL (Cat #: KT03961-1-302.7)
- Samples: Whole brain from three 10-weeks old mice and four 2-week old mice
- Buffer: 100mg/ml of 0.1M K_2PO_4, pH 7.0 containing 1mM EDTA and 10µM indomethacin

/ PROTOCOL

Whole brains from healthy mice were obtained following standard practices and stored at -80°C until use

Samples were thawed on ice and were homogenized in 1mL homogenization buffer in 7mL Precellys® tubes containing 2.8 mm ceramic beads: 2 cycles of 30 sec at 5000 rpm, with a 30 sec break

100 mg of homogenate was used for the extractions and the quantification was performed with LC-MS/MS and IA-MS instrumentation*.

Baker, M. Nature, 521(7552), 274-276 (2015); Handelsman, D.J. & Wortofsky, L.J. Clin. Endocrin. Metab. 98(10), 3971-3973 (2013).

/ RESULTS

Table 1. The comparison of Leukotriene concentrations (units) isolated from mice brain homogenates as measured by the indicated method between two extraction methods.

	LTE_4 (pg/mg)		LTD_4 (pg/mg)		LTC_4 (pg/mg)		LTB_4 (pg/mg)	
Brain Sample	SPE	IA	SPE	IA	SPE	IA	SPE	IA
B11	0.059	0.058	0.092	0.103	0.039	0.068	<LOQ	0.032
B12	0.029	0.038	0.083	0.063	0.012	0.026	<LOQ	0.072
B13	0.028	0.034	0.042	0.034	0.024	0.053	<LOQ	0.033
B14	0.027	0.030	0.063	0.045	0.021	0.016	<LOQ	0.023
Average	0.036	0.040	0.070	0.061	0.024	0.041	0.000	0.040
Mean Difference	9.85%		-14.46%		40.66%		100.00%	

Table 2. The concentrations (pg/mg) of Leukotriene levels observed in different mouse brain samples. BQL = Below Quantitation Limit.

Sample ID	LTB_4	LTC_4	LTD_4	LTE_4
p14_WT-1	0.032	0.068	0.103	0.058
p14_WT-2	0.072	0.026	0.063	0.038
p14_WT-3	0.033	0.053	0.034	0.034
p14_WT-4	0.023	BQL	0.045	0.030
AVG	0.040	0.041	0.061	0.040
STD DEV	0.022	0.024	0.030	BQL
adult_WT-1	0.137	0.066	0.109	0.073
adult_WT-2	0.084	0.089	0.069	0.076
adult_WT-3	0.188	0.038	0.107	0.087
AVG	0.136	0.064	0.095	0.079
STD DEV	0.052	0.026	0.023	BQL

/ CUSTOMER

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https://www.caymanchem.com/cms/caymanchem/Literature/800164.pdf

/ CONCLUSION

The study demonstrated the efficiency of Precellys® Evolution homogenizer for whole mice brain tissue homogenization. Thanks to the flexibility offers by the 7 ml Precellys® Lysing kit format, the grinding of the whole mice brain tissue can be performed within one tube.

The combination of the efficient samples preparation with the Precellys® Evolution and an optimized extraction method allowed to better understand isolation efficiency and quantification of Leukotrienes in brain tissue.

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