Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information

Dynamic Eicosanoid Responses Upon Different Inhibitor and Combination Treatments on the Arachidonic Acid Metabolic Network

Contents:

Table S8. Eicosanoid levels in response to A23187 with 5-LOX/PGES inhibitor zileuton/MF63.

Table S9. Eicosanoid levels in response to A23187 with LTA4H/COX inhibitor SC-22716/indomethacin.

Table S10. Eicosanoid levels in response to A23187 with LTA4H/PGES inhibitor SC-22716/MF63.

Table S1. Validation of the LC–SRM–MS method for the determination of eicosanoids.

Analytes	Linear range (10^{-6} Mol L^{-1})	Detection limit (10^{-7} Mol L^{-1})	Regression equations	R^2
6-keto $PGF_{1\alpha}$	1 – 100.0	2.70	Q = 85.52C + 0.7352	0.9906
TXB_2	0.1 – 100.0	0.025	Q = 42.25C – 10.16	0.9985
$PGF_{2\alpha}$	0.1 – 50.0	0.22	Q = 80.94C – 0.7741	0.9983
PGE_2	0.05 – 50.0	0.012	Q = 19.33C – 6.684	0.9969
PGD_2	0.05 – 50.0	0.18	Q = 5.251C + 2.623	0.9946
LTE_4	0.05 – 50.0	0.25	Q = 56.25C + 34.77	0.9924
LTB_4	0.01 – 100.0	0.010	Q = 91.26C + 35.34	0.9924
15-HETE	0.05 – 50.0	0.022	Q = 99.81C – 2.553	0.9905
12-HETE	0.05 – 50.0	0.018	Q = 35.39C + 804.6	0.9852
5-HETE	0.05 – 50.0	0.035	Q = 119.2C – 1.359	0.9993
AA	0.1 – 1000.0	0.18	Q = 10.46C + 219.6	0.9854

Table S2. Eicosanoid levels in response to A23187 without inhibitor.[a]

Time (min)	Eicosanoid levels (μM)						
	5-HETE	12-HETE	15-HETE	LTB_4	PGE_2	TXB_2	AA
0 min	0.05±0.01	50.9±17.8	0.27±0.09	0.15±0.09	0.13±0.06	0.46±0.15	1492±55
10 min	0.39±0.18	87.3±56.8	0.79±0.26	0.30±0.18	0.18±0.11	0.46±0.08	1633±197
20 min	0.29±0.13	86.4±36.3	1.02±0.33	0.18±0.04	0.47±0.18	0.64±0.33	1595±233
30 min	0.57±0.15	123±45	1.11±0.43	0.54±0.30	0.44±0.10	0.67±0.28	1611±301
40 min	0.65±0.14	145±65	2.27±0.55	2.09±0.54	0.57±0.12	0.85±0.30	1548±139
50 min	0.70±0.21	153±64	2.17±0.60	1.66±0.60	0.46±0.05	1.28±0.48	1558±205
60 min	0.45±0.12	163±81	2.28±0.30	1.31±0.30	0.31±0.16	1.48±0.21	1443±74
80 min	0.54±0.17	178±54	2.16±0.22	1.31±0.23	0.30±0.05	0.87±0.30	1549±248
100 min	0.31±0.23	205±61	3.04±0.16	1.24±0.16	0.38±0.09	0.55±0.18	1450±179
120 min	0.22±0.12	294±77	2.94±0.30	0.89±0.30	0.36±0.05	0.37±0.10	1555±245

[a] Each data expressed as the mean ± S.E. and representative of at least three independent experiments at each time point. When the concentration of eicosanoid was over the highest calibration point, the sample was diluted with methanol and reanalyzed.

Table S3. Eicosanoid levels in response to A23187 with COX inhibitor indomethacin.[a]

Time	Eicosanoid levels (μM)						
(min)	5-HETE	12-HETE	15-HETE	LTB_4	PGE_2	TXB_2	AA
0 min	0.38±0.04	91.4±9.0	0.43±0.06	0.40±0.07	0.07±0.02	0.04±0.01*	1552±100
10 min	0.45±0.06	148±15	0.66±0.06	0.86±0.14	0.07±0.02	0.05±0.01*	1633±46
20 min	0.55±0.06	133±17	0.70±0.08	1.16±0.15	0.06±0.03	0.07±0.00*	1690±46
30 min	0.87±0.04	151±12	1.15±0.17	1.41±0.09	0.15±0.07	0.08±0.01*	1550±58
40 min	1.04±0.10	192±6	1.44±0.18	2.05±0.20	0.20±0.05	0.11±0.01	1717±47
50 min	1.09±0.16	237±15	2.15±0.16	2.43±0.10	0.24±0.04	0.10±0.01	1809±45
60 min	0.97±0.09	328±8	2.62±0.20	3.05±0.20	0.14±0.02	0.08±0.02*	1755±52
80 min	0.87±0.06	368±7	2.86±0.18	3.64±0.35	0.12±0.02	0.09±0.01*	1714±45
100 min	0.63±0.05	356±11	3.39±0.19	2.96±0.27	0.17±0.03	0.06±0.01*	1985±44
120 min	0.86±0.07	333±15	3.16±0.22	2.64±0.17	0.13±0.02	0.04±0.01*	2325±194

[a] Each data expressed as the mean ± S.E. and representative of at least three independent experiments at each time point. When the concentration of eicosanoid was over the highest calibration point, the sample was diluted with methanol and reanalyzed.

* Concentration detected below the range of quantitable concentration.

Table S4. Eicosanoid levels in response to A23187 with PGES inhibitor MF63.[a]

Time (min)	Eicosanoid levels (μM)						
	5-HETE	12-HETE	15-HETE	LTB_4	PGE_2	TXB_2	AA
0 min	0.45±0.13	31.4±2.7	1.43±0.43	0.81±0.11	0.06±0.03	0.62±0.08*	641±88
10 min	0.65±0.09	39.4±6.7	3.31±0.23	1.03±0.05	0.08±0.04	0.79±0.21*	1369±126
20 min	0.66±0.09	62.8±8.7	3.03±0.16	0.91±0.02	0.07±0.01	1.04±0.17	1423±64
30 min	0.74±0.05	117±15	3.10±0.79	1.01±0.05	0.07±0.01	1.08±0.20	1139±144
40 min	1.01±0.18	114±10	4.17±0.33	1.33±0.12	0.04±0.01*	1.36±0.22	1757±167
50 min	0.97±0.21	127±34	3.18±0.14	1.76±0.08	0.04±0.01*	1.68±0.18	2032±197
60 min	0.68±0.13	151±15	2.84±0.72	1.75±0.39	0.05±0.01	1.48±0.36	1693±60
80 min	0.84±0.15	146±11	2.57±0.73	2.02±0.23	0.06±0.02	1.14±0.13	2225±20
100 min	0.75±0.09	149±7	2.57±0.53	1.52±0.13	0.06±0.03	0.91±0.18*	1727±133
120 min	0.67±0.03	167±30	3.43±0.22	1.63±0.21	0.05±0.02	0.72±0.23*	2103±332

[a] Each data expressed as the mean ± S.E. and representative of at least three independent experiments at each time point. When the concentration of eicosanoid was over the highest calibration point, the sample was diluted with methanol and reanalyzed.

* Concentration detected below the range of quantitable concentration.

Table S5. Eicosanoid levels in response to A23187 with 5-LOX inhibitor zileuton.[a]

Time (min)	Eicosanoid levels (μM)						
	5-HETE	12-HETE	15-HETE	LTB_4	PGE_2	TXB_2	AA
0 min	0.04±0.02*	18.4±3.7	0.60±0.33	0.25±0.08	0.69±0.29	5.45±1.96	1581±121
10 min	0.06±0.01	28.9±9.4	1.31±0.36	0.14±0.03	0.86±0.27	4.75±1.56	1612±147
20 min	0.05±0.03	71.4±14.8	1.27±0.42	0.24±0.04	0.80±0.20	6.55±1.22	1556±56
30 min	0.04±0.01*	85.9±26.3	1.81±0.26	0.15±0.11	1.30±0.45	4.39±1.84	1460±52
40 min	0.05±0.02	89.8±33.0	2.39±0.38	0.23±0.13	1.27±0.28	4.61±1.63	1407±118
50 min	0.05±0.03	106±45	2.19±0.52	0.29±0.04	1.36±0.41	4.90±1.36	1500±86
60 min	0.09±0.01	137±46	3.22±0.79	0.41±0.13	1.63±0.39	6.96±1.53	1639±46
80 min	0.14±0.03	161±20	2.88±0.53	0.47±0.15	1.26±0.26	9.66±1.28	1475±89
100 min	0.10±0.06	155±21	2.92±0.79	0.33±0.03	1.19±0.42	4.83±1.39	1575±77
120 min	0.15±0.02	187±47	2.57±0.33	0.16±0.06	1.12±0.45	4.14±1.15	1594±121

[a] Each data expressed as the mean ± S.E. and representative of at least three independent experiments at each time point. When the concentration of eicosanoid was over the highest calibration point, the sample was diluted with methanol and reanalyzed.

* Concentration detected below the range of quantitable concentration.

Table S6. Eicosanoid levels in response to A23187 with LTA4H inhibitor SC-22716.[a]

Time (min)	Eicosanoid levels (μM)						
	5-HETE	12-HETE	15-HETE	LTB_4	PGE_2	TXB_2	AA
0 min	0.58±0.04	71.3±6.3	1.19±0.13	0.20±0.02	0.37±0.02	1.69±0.09	1089±145
10 min	0.62±0.04	75.8±2.3	1.76±0.18	0.46±0.10	0.42±0.08	1.57±0.12	1170±380
20 min	0.68±0.20	98.7±8.3	1.66±0.50	0.43±0.15	0.27±0.12	2.02±0.45	1153±131
30 min	0.49±0.11	96.4±4.3	1.74±0.09	0.42±0.05	0.81±0.08	2.79±0.64	1168±132
40 min	0.39±0.14	116±13	2.41±0.23	0.40±0.07	0.61±0.07	3.17±0.59	1030±268
50 min	0.29±0.02	124±14	3.17±0.20	0.40±0.04	0.64±0.10	3.54±0.32	1353±238
60 min	0.32±0.20	151±15	2.84±0.24	0.33±0.11	0.83±0.05	4.37±0.26	1318±54
80 min	0.35±0.03	172±4	3.22±0.59	0.18±0.06	0.58±0.05	3.13±0.39	1769±322
100 min	0.26±0.04	168±8	2.81±0.47	0.16±0.03	0.28±0.08	2.50±0.38	1588±166
120 min	0.18±0.03	163±7	2.31±0.28	0.08±0.05	0.30±0.09	1.53±0.33	1684±313

[a] Each data expressed as the mean ± S.E. and representative of at least three independent experiments at each time point. When the concentration of eicosanoid was over the highest calibration point, the sample was diluted with methanol and reanalyzed.

Table S7. Eicosanoid levels in response to A23187 with 5-LOX/COX inhibitor zileuton/indomethacin.[a]

Time (min)	Eicosanoid levels (μM)						
	5-HETE	12-HETE	15-HETE	LTB_4	PGE_2	TXB_2	AA
0 min	0.27±0.06	30.0±3.2	2.38±0.45	0.27±0.05	0.31±0.03	0.09±0.01*	1412±64
10 min	0.14±0.03	18.7±4.1	3.82±0.35	0.30±0.06	0.30±0.07	0.05±0.04*	1600±69
20 min	0.17±0.03	65.5±4.1	4.41±0.36	0.18±0.07	0.43±0.07	0.47±0.08	1508±61
30 min	0.17±0.04	69.3±4.4	6.73±0.51	0.85±0.05	0.50±0.10	0.67±0.11	1949±87
40 min	0.30±0.04	132±9	6.37±0.58	1.49±0.06	0.39±0.08	0.84±0.10	1168±129
50 min	0.28±0.04	122±4	4.29±0.40	1.06±0.12	0.22±0.12	0.65±0.07	1667±50
60 min	0.44±0.03	131±7	3.64±0.51	1.01±0.04	0.22±0.09	0.86±0.05	1699±25
80 min	0.45±0.04	195±7	3.21±0.62	0.71±0.07	0.23±0.06	0.63±0.06	1228±25
100 min	0.34±0.04	159±6	3.32±0.34	0.64±0.05	0.18±0.08	0.79±0.08	1756±69
120 min	0.22±0.04	166±8	3.67±0.55	0.59±0.09	0.23±0.06	0.77±0.09	1886±43

[a] Each data expressed as the mean ± S.E. and representative of at least three independent experiments at each time point. When the concentration of eicosanoid was over the highest calibration point, the sample was diluted with methanol and reanalyzed.

* Concentration detected below the range of quantitable concentration.

Table S8. Eicosanoid levels in response to A23187 with 5-LOX/PGES inhibitor zileuton/MF63.[a]

Time (min)	Eicosanoid levels (μM)						
	5-HETE	12-HETE	15-HETE	LTB_4	PGE_2	TXB_2	AA
0 min	0.17±0.04	7.82±3.45	4.85±0.55	0.84±0.10	0.04±0.01*	0.47±0.09	1029±174
10 min	0.35±0.07	3.56±1.59	5.96±0.85	0.41±0.23	0.03±0.02*	0.48±0.15	977±100
20 min	0.49±0.12	10.3±2.6	4.35±0.45	0.59±0.11	0.03±0.01*	0.52±0.17	872±102
30 min	0.37±0.14	11.4±1.3	8.78±0.44	0.47±0.17	0.04±0.01*	0.88±0.07	1173±188
40 min	0.18±0.11	9.46±3.51	7.47±1.01	0.33±0.20	0.04±0.01*	1.25±0.15	1161±249
50 min	0.19±0.06	10.2±1.4	6.72±0.77	0.30±0.13	0.06±0.01	1.73±0.23	970±62
60 min	0.25±0.05	18.9±301	11.1±1.8	0.83±0.14	0.03±0.01*	1.09±0.08	1948±55
80 min	0.18±0.08	15.6±1.1	8.21±1.35	0.73±0.09	0.03±0.01*	0.73±0.10	1821±93
100 min	0.29±0.09	28.2±3.1	10.48±1.9	0.48±0.19	0.03±0.01*	0.48±0.16	1484±210
120 min	0.22±0.12	32.0±5.3	12.68±1.4	0.42±0.13	0.05±0.01	0.44±0.20	1559±296

[a] Each data expressed as the mean ± S.E. and representative of at least three independent experiments at each time point. When the concentration of eicosanoid was over the highest calibration point, the sample was diluted with methanol and reanalyzed.

* Concentration detected below the range of quantitable concentration.

Table S9. Eicosanoid levels in response to A23187 with LTA4H/COX inhibitor SC-22716/ indomethacin.[a]

Time (min)	Eicosanoid levels (μM)						
	5-HETE	12-HETE	15-HETE	LTB_4	PGE_2	TXB_2	AA
0 min	0.32±0.08	100±18	0.69±0.11	0.07±0.01	0.04±0.02*	0.11±0.04	650±50
10 min	0.23±0.12	112±14	0.96±0.04	0.21±0.11	0.03±0.01*	0.18±0.02	620±25
20 min	0.29±0.07	164±28	1.11±0.19	0.21±0.07	0.03±0.00*	0.20±0.01	652±74
30 min	0.20±0.04	216±16	1.10±0.26	0.32±0.17	0.03±0.00*	0.25±0.02	953±204
40 min	0.35±0.05	271±27	1.56±0.12	0.56±0.09	0.03±0.01*	0.21±0.02	1245±112
50 min	0.39±0.09	282±15	1.90±0.16	0.62±0.11	0.03±0.00*	0.14±0.02	1572±353
60 min	0.46±0.06	354±25	2.23±0.24	0.44±0.19	0.03±0.01*	0.12±0.01	1286±49
80 min	0.40±0.06	348±26	2.45±0.32	0.51±0.06	0.03±0.01*	0.09±0.01*	1353±222
100 min	0.44±0.11	296±21	3.04±0.22	0.80±0.14	0.04±0.01*	0.09±0.01*	1478±199
120 min	0.46±0.07	302±38	2.74±0.24	0.39±0.12	0.04±0.01*	0.07±0.01*	1159±57

[a] Each data expressed as the mean ± S.E. and representative of at least three independent experiments at each time point. When the concentration of eicosanoid was over the highest calibration point, the sample was diluted with methanol and reanalyzed.

* Concentration detected below the range of quantitable concentration.

Table S10. Eicosanoid levels in response to A23187 with LTA4H/PGES inhibitor SC-22716/ MF63.[a]

Time (min)	Eicosanoid levels (μM)						
	5-HETE	12-HETE	15-HETE	LTB_4	PGE_2	TXB_2	AA
0 min	0.15±0.01	60.8±9.2	0.75±0.07	0.17±0.04	0.05±0.01	0.41±0.08	1607±76
10 min	0.21±0.03	72.0±10.8	1.49±0.25	0.21±0.06	0.04±0.01*	0.39±0.05	1430±85
20 min	0.33±0.06	88.6±13.8	2.53±0.34	0.38±0.09	0.04±0.01*	0.62±0.08	1258±243
30 min	0.50±0.09	98.3±18.1	1.97±0.20	0.44±0.08	0.05±0.01	0.57±0.14	1353±300
40 min	0.48±0.12	104±15	2.65±0.32	0.49±0.09	0.04±0.01*	1.03±0.09	1735±33
50 min	0.39±0.04	110±13	2.53±0.52	0.35±0.05	0.02±0.01*	1.66±0.21	1129±159
60 min	0.65±0.06	123±25	2.83±0.41	0.30±0.02	0.02±0.00*	1.34±0.10	1508±108
80 min	0.71±0.18	131±36	2.44±0.65	0.66±0.11	0.05±0.01	0.99±0.06	1065±68
100 min	0.59±0.03	185±10	2.85±0.40	0.70±0.13	0.03±0.01*	0.78±0.12	912±77
120 min	0.73±0.14	201±24	3.29±0.76	0.82±0.11	0.03±0.00*	0.52±0.08	1322±202

[a] Each data expressed as the mean ± S.E. and representative of at least three independent experiments at each time point. When the concentration of eicosanoid was over the highest calibration point, the sample was diluted with methanol and reanalyzed.

* Concentration detected below the range of quantitable concentration.