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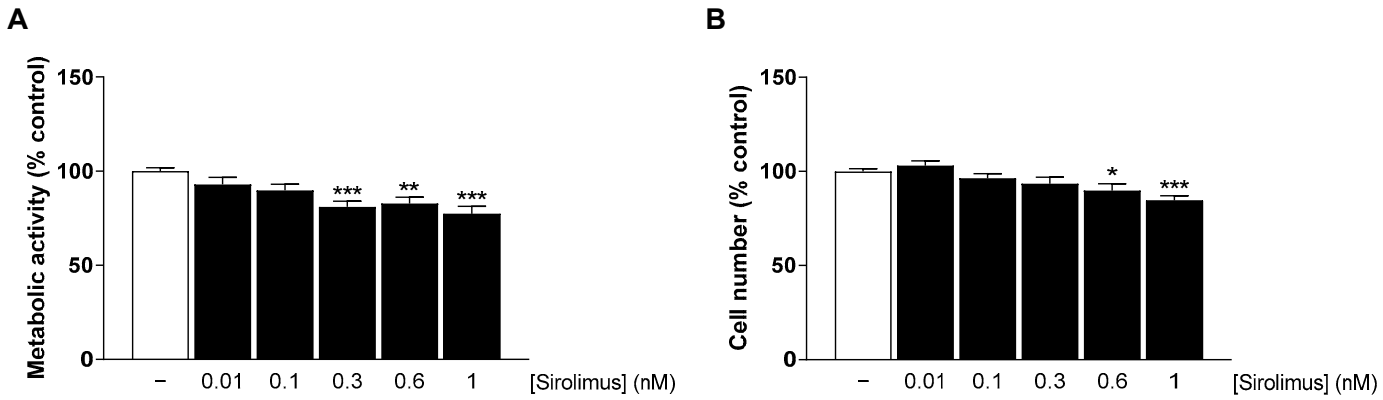
MDPI

Supplementary Material

Non-Psychoactive Phytocannabinoids Inhibit Inflammation-Related Changes of Human Coronary Artery Smooth Muscle and Endothelial Cells

Elisa Teichmann, Elane Blessing and Burkhard Hinz *

Institute of Pharmacology and Toxicology, Rostock University Medical Center, Schillingallee 70, 18057 Rostock, Germany; elisa.teichmann@web.de (E.T.); elane.blessing@med.uni-rostock.de (E.B.)
* Correspondence: burkhard.hinz@med.uni-rostock.de; Tel.: +49-381-494-5770



Supplementary Figure S1: Effect of sirolimus on metabolic activity and cell number of HCAEC. HCAEC were incubated with increasing concentrations of sirolimus or with vehicle for 24 h. Thereafter, metabolic activity was determined by WST-1 assay (**A**) and cell number by crystal violet staining (**B**). Vehicle-treated cells were used as controls (100%). Data are presented as means ± SEM of $n = 12$ (4 independent experiments). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ vs. vehicle control; one-way ANOVA plus Dunnett post hoc test.

Supplementary Table S1: Effect of cannabinoid receptor antagonists on metabolic activity and cell number of HCAEC under basal, IL-1β-, or LPS-stimulated conditions. HCAEC were preincubated with 1 μM AM251, 1 μM AM630, 1 μM capsazepine, or vehicle for 1 h, followed by the addition of 10 ng/mL IL-1β, 1 μg/mL LPS, or vehicle and a further 24 h incubation with the respective compounds. Subsequently, metabolic activity was determined by WST-1 assay and cell number by crystal violet staining. Cells treated with vehicle were used as control (set as 100%). Data are presented as means ± SEM of $n = 9$ (3 independent experiments). * $p \leq 0.05$ vs. vehicle control; one-way ANOVA plus Bonferroni post hoc test.

Treatment group	Metabolic activity (%)	Cell number (%)	Treatment group	Metabolic activity (%)	Cell number (%)
Vehicle	100.0 ± 0.9	100.0 ± 2.1	Vehicle	100.0 ± 1.9	100.0 ± 2.0
IL-1β	97.9 ± 2.5	104.2 ± 4.8	LPS	107.3 ± 3.5	106.8 ± 3.1
AM251	107.5 ± 1.6	103.7 ± 1.9	AM251	113.2 ± 2.3	103.5 ± 3.4
AM251 + IL-1β	106.2 ± 2.0	105.0 ± 4.1	AM251 + LPS	118.3 ± 5.0	108.5 ± 2.9
AM630	103.1 ± 2.2	101.5 ± 3.1	AM630	110.0 ± 2.6	111.5 ± 2.6
AM630 + IL-1β	94.5 ± 3.0	104.3 ± 4.1	AM630 + LPS	113.0 ± 3.9	111.5 ± 2.4
AM251 + AM630	105.2 ± 1.6	97.0 ± 6.3	AM251 + AM630	115.4 ± 4.8	107.2 ± 4.1
AM251 + AM630 + IL-1β	103.4 ± 3.4	107.3 ± 4.5	AM251 + AM630 + LPS	122.1 ± 6.9*	114.3 ± 1.7
Capsazepine	108.5 ± 1.6	103.4 ± 5.2	Capsazepine	119.9 ± 8.0	109.1 ± 1.8
Capsazepine + IL-1β	99.1 ± 3.1	110.3 ± 3.8	Capsazepine + LPS	113.9 ± 5.4	107.9 ± 4.3

Supplementary Table S2: Effect of MAPK inhibitors on metabolic activity and cell number of HCAEC under basal, IL-1β-, or LPS-stimulated conditions. HCAEC were preincubated with 10 μM SB203580 (p38 MAPK inhibitor), 10 μM PD98059 (p42/44 MAPK inhibitor), 10 μM SP600125 (JNK inhibitor), or vehicle for 1 h, followed by the addition of 10 ng/mL IL-1β, 1 μg/mL LPS, or vehicle and a further 24 h incubation with the respective compounds. Subsequently, metabolic activity was determined by WST-1 assay and cell number by crystal violet staining. Cells treated with vehicle were used as control (set as 100%). Data are presented as means ± SEM of $n = 9$ (3 independent experiments). * $p \leq 0.05$ vs vehicle control; # $p \leq 0.05$ vs. stimulated control; one-way ANOVA plus Bonferroni post hoc test.

Treatment group	Metabolic activity (%)	Cell number (%)	Treatment group	Metabolic activity (%)	Cell number (%)
Vehicle	100.0 ± 1.5	100.0 ± 1.7	Vehicle	100.0 ± 1.3	100.0 ± 2.2
IL-1β	98.4 ± 3.6	103.2 ± 2.7	LPS	95.4 ± 1.4	98.0 ± 3.7
SB203580	96.9 ± 1.9	112.4 ± 3.6	SB203580	88.9 ± 2.3*	105.3 ± 2.8
SB203580 + IL-1β	84.6 ± 3.6*	112.1 ± 3.2	SB203580 + LPS	84.1 ± 1.6*#	105.7 ± 2.8
PD98059	87.2 ± 1.9	94.8 ± 2.9	PD98059	74.8 ± 2.6*	88.7 ± 1.9
PD98059+ IL-1β	80.5 ± 1.8*#	103.5 ± 2.5	PD98059 + LPS	72.7 ± 2.2*#	94.1 ± 4.5
SP600125	80.1 ± 5.5*	74.1 ± 4.9*	SP600125	67.0 ± 1.9*	68.2 ± 2.2*
SP600125 + IL-1β	89.5 ± 5.2	75.7 ± 2.4*#	SP600125 + LPS	69.1 ± 1.9*#	74.7 ± 4.7*#

Supplementary Table S3: Effect of NF-κB inhibitor BAY 11-7082 (BAY) on metabolic activity and cell number of HCAEC under basal, IL-1β-, or LPS-stimulated conditions. IL-1β- and LPS-stimulated HCAEC metabolic activity and cell number. HCAEC were preincubated with increasing concentrations of BAY 11-7082 or vehicle for 1 h, followed by the addition of 10 ng/mL IL-1β, 1 μg/mL LPS, or vehicle and a further 24 h incubation with the respective compounds. Subsequently, metabolic activity was determined by WST-1 assay and cell number by crystal violet staining. Cells treated with vehicle were used as control (set as 100 %). Data are presented as means ± SEM of $n = 9$ (3 independent experiments). * $p \leq 0.05$ vs. vehicle control; # $p \leq 0.05$ vs. stimulated control; one-way ANOVA plus Bonferroni post hoc test.

Treatment group	Metabolic activity (%)	Cell number (%)	Treatment group	Metabolic activity (%)	Cell number (%)
Vehicle	100.0 ± 1.7	100.0 ± 3.0	Vehicle	100.0 ± 1.6	100.0 ± 1.8
IL-1β	104.2 ± 2.3	104.9 ± 2.6	LPS	99.7 ± 1.9	100.6 ± 2.9
0.01 μM BAY	107.8 ±2.3	100.2 ± 5.3	0.01 μM BAY	100.5 ± 0.9	111.2 ± 8.3
0.01 μM BAY + IL-1β	110.4 ± 2.1	117.9 ± 5.3	0.01 μM BAY + LPS	104.1 ± 1.6	104.3 ± 2.6
0.1 μM BAY	116.4 ± 2.0*	105.1 ± 3.1	0.1 μM BAY	112.7 ± 2.5	107.8 ± 2.5
0.1 μM BAY + IL-1β	118.9 ± 2.2*	116.4 ± 5.2	0.1 μM BAY + LPS	113.0 ± 2.7	105.1 ± 3.3
1 μM BAY	132.3 ± 2.5*	108.2 ± 5.1	1 μM BAY	132.4 ± 3.8*	105.5 ± 2.7
1 μM BAY + IL-1β	129.8 ± 6.2*#	114.2 ± 3.7	1 μM BAY + LPS	136.9 ± 4.1*#	108.9 ± 3.1
5 μM BAY	25.3 ± 6.5*	37.6 ± 6.6*	5 μM BAY	21.1 ± 5.9*	42.7 ± 4.1*
5 μM BAY + IL-1β	26.0 ± 6.4*#	40.0 ± 4.8*#	5 μM BAY + LPS	24.2 ± 5.4*#	47.3 ± 5.7*#
10 μM BAY	1.7 ± 0.3*	23.3 ± 5.5*	10 μM BAY	1.0 ± 0.5*	32.9 ± 6.1*
10 μM BAY + IL-1β	1.9 ± 0.4*#	23.0 ± 5.8*#	10 μM BAY + LPS	1.7 ± 0.6*#	42.4 ± 7.5*#

Supplementary Table S4: Effect of HDAC inhibitor trichostatin A (TSA) on metabolic activity and cell number of HCAEC under basal, IL-1β-, or LPS-stimulated conditions. HCAEC were preincubated with increasing concentrations of TSA or vehicle for 1 h, followed by the addition of 10 ng/mL IL-1β, 1 μg/mL LPS, or vehicle and a further 24 h incubation with the respective compounds. Subsequently, metabolic activity was determined by WST-1 assay and cell number by crystal violet staining. Cells treated with vehicle were used as control (set as 100 %). Data are presented as means ± SEM of $n = 9$ (3 independent experiments). * $p \leq 0.05$ vs. vehicle control; # $p \leq 0.05$ vs. stimulated control; one-way ANOVA plus Bonferroni post hoc test.

Treatment group	Metabolic activity (%)	Cell number (%)	Treatment group	Metabolic activity (%)	Cell number (%)
Vehicle	100.0 ± 1.8	100.0 ± 1.7	Vehicle	100.0 ± 1.4	100.0 ± 3.2
IL-1β	102.8 ± 2.3	99.6 ± 4.6	LPS	100.0 ± 2.2	105.6 ± 3.3
0.01 μM TSA	94.5 ± 1.6	100.4 ± 3.1	0.01 μM TSA	99.3 ± 2.6	107.1 ± 2.6
0.01 μM TSA + IL-1β	102.5 ± 1.4	101.4 ± 3.0	0.01 μM TSA + LPS	97.4 ± 2.0	103.0 ± 3.4
0.1 μM TSA	94.1 ± 2.2	94.5 ± 2.8	0.1 μM TSA	92.1 ± 1.1	95.9 ± 2.0
0.1 μM TSA + IL-1β	102.1 ± 2.6	95.1 ± 3.0	0.1 μM TSA + LPS	97.7 ± 3.0	99.2 ± 3.1
1 μM TSA	74.4 ± 3.2*	74.9 ± 2.6*	1 μM TSA	76.5 ± 3.4*	77.2 ± 1.9*
1 μM TSA + IL-1β	76.2 ± 4.6*#	71.0 ± 4.3*#	1 μM TSA + LPS	73.1 ± 3.2*#	70.7 ± 1.7*#
5 μM TSA	67.6 ± 3.3*	67.2 ± 1.8*	5 μM TSA	71.2 ± 3.6*	68.8 ± 2.8*
5 μM TSA + IL-1β	62.0 ± 3.6*#	58.4 ± 4.6*#	5 μM TSA + LPS	64.5 ± 4.4*#	62.8 ± 2.5*#
10 μM TSA	67.1 ± 4.1*	64.5 ± 1.9*	10 μM TSA	70.1 ± 4.3*	72.4 ± 4.9*
10 μM TSA + IL-1β	58.3 ± 3.3*#	57.0 ± 3.4*#	10 μM TSA + LPS	64.5 ± 3.8*#	62.3 ± 3.3*#