

Muscarinic receptor subtype-specific effects on cigarette smoke-induced inflammation in mice

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Tables

Table E1: Average weight of WT, M₁^{-/-}, M₂^{-/-} and M₃^{-/-} mice before and after one week of air or CS exposure

	Air exposed		Smoke exposed	
	Before	After	Before	After
WT mice	27.5 (±0.62)	27.3 (±0.73)	26.0 (±0.53)	25.7 (±0.67)
M₁^{-/-} mice	26.3 (±0.55)	26.0 (±0.54)	22.7 (±0.85)	22.6 (±0.93)
M₂^{-/-} mice	25.8 (±0.56)	25.4 (±0.47)	27.6 (±0.38)	27.3 (±0.29)
M₃^{-/-} mice	22.8 (±0.53)	23.7 (±0.71)	20.3 (±0.58)	21.0 (±0.41)

Table E2: Primers used for qRT-PCR analysis. CHT1: high-affinity choline transporter 1; CTL: choline transporter-like protein 1; ChAT: choline acetyltransferase; AChE: acetylcholinesterase

Gene	Primer sequence	NCBI accession number
Muscarinic M₁ receptor	Forward – AGAAGAGGCTGCCACAGGTA Reverse – CAGACCCCACCTGGACTTTA	NM_001112697
Muscarinic M₂ receptor	Forward – GAATGGGGATGAAAAGCAGA Reverse – GCAGGGTTGATGGTGCTATT	NM_203491
Muscarinic M₃ receptor	Forward – CACAGCCAAGACCTCTGACA Reverse – ATGATGTTGTAGGGGGTCCA	NM_033269
CHT1	Forward – GGTTGGAGGAGGCTACATCA Reverse – TATCCCTTGGAACGCATAGG	AJ401467

CTL1	Forward – GGCAGTGCTGTTCAGAATGA Reverse – ATCTGCTGACAGGCGAGAAT	NM_133891
ChAT	Forward – GGCTGCAAAGAGACCTCATC Reverse – GCTCTTTCCTTTGCTGTTGG	NM_009891
AChE	Forward – CCTGGGTTTGGAGGGTACTGA Reverse – GGTTCCCACTCGGTAGTTCA	NM_009599
Fibronectin	Forward – ACCACCCAGAACTACGATGC Reverse – GGAACGTGTCGTTACATTG	NM_010233
Collagen Iα1	Forward – CACCCTCAAGAGCCTGAGTC Reverse – GTTCGGGCTGATGTACCAGT	NM_007742
MUC5AC	Forward – GAGATGGAGGATCTGGGTCA Reverse – GCAGAAGCAGGGAGTGGTAG	NM_010844
18S	Forward – AAACGGCTACCACATCCAAG Reverse – CCTCCAATGGATCCTCGTTA	NR_003278

Table E3: Primers used for genotyping.

Gene	Primer sequence
M1-S1	5'-CCAACATCACCGTCTTGGCAC-3'
M1-A1	5'-AGTGCCAATGATGAGATCAGC-3'
M2-A6	5'-GCTATTACCAGTCCTTACAAGACA-3'
M2-B5	5'-CCAGAGGATGAAGGAAAGAACC-3'
M3-A3	5'-AAGACCACAGTAGCAGTG-3'

M3-B	5'-CTCTCTACATCCATAGTCCC-3'
NEO-1	5'-CAGCTCATTCCCTCCCACTCATGAT-3'
M3-NEO9	5'-TGGATGTGGAATGTGTGCGAGG-3'

Figures

Figure E1. Muscarinic receptor expression in lung tissue and BAL cells. Mice were treated as described in Figure 1. Sixteen hours after the last smoke exposure lung tissue and bronchoalveolar lavage fluid (BALF) were harvested. Gene expression in lung tissue homogenates (grey bars) and in BAL cells (black bars) was analyzed (n=3-5 mice per group). Ct values corrected for 18S are depicted, expressed as mean \pm s.e. of the mean. Note that low Ct values mean high expression levels. Muscarinic M₁ receptor (M1R), M₂ receptor (M2R) and M₃ receptor (M3R). Data were analyzed using two-way ANOVA; F=16.63; DF=1; p<0.001 for the comparison lung tissue vs. BAL cells. F=11.71; DF=2; p<0.001 for the comparison of muscarinic receptor subtypes. F=12.126; DF=2; p<0.001 for the interaction. Individual comparisons were made using a Student-Newman-Keuls multiple comparisons post-hoc test; *** p < 0.001 (adjusted p-value). Statistics were performed on log-transformed data.

