Matrix metalloproteinase-1 and skin ageing in smokers

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Smokers look older than non-smokers of the same age. We have compared the concentrations of mRNA for matrix metalloproteinase 1 (MMP-1) in the buttock skin of smokers and non-smokers with quantitative real-time polymerase chain reactions. MMP-1 degrades collagen, which accounts for at least 70% of the dry weight of dermis. We report significantly more MMP-1 mRNA in the skin of smokers than non-smokers whereas no difference was seen for the tissue inhibitor of metalloproteinases 1 (TIMP-1) or the housekeeping gene *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase). We suggest that smoking-induced MMP-1 might be important in the skin-ageing effects of tobacco smoking.

Solar ultraviolet radiation[1] and smoking[2] are known to have an ageing effect on human skin, especially in the facial region. Ultraviolet induction of matrix metalloproteinases (MMPs) could mediate the effect of sunlight on skin ageing[1] (photoageing). MMPs are zinc-dependent proteases

Target	Amplicon	Primer/probe	Sequence (5'-3')
MMP-1	73 bp	MMP-1 forward	AGCTAGCTCAGGATGACATTGATG
		MMP-1 probe	CATCCAAGCCATATATGGACGTTCCCAAA
		MMP-1 reverse	CCGATGGGCTGGACAGG
TIMP-1	63 bp	TIMP-1 forward	TGGTGTCCCCACGAACTTG
		TIMP-1 probe	CCCTGATGACGAGGTCGGAATTGC
		TIMP-1 reverse	CACCCACAGACGGCCTTC
GAPDH	76 bp	GAPDH forward	TGGGTGTGAACCATGAGAAG
		GAPDH probe	CCTCAAGATCATCAGCAATGCCTCC
		GAPDH reverse	GCTAAGCAGTTGGTGGTGC

The TaqMan RT-PCR method measures PCR product accumulation via an oligonucleotide probe labelled with a fluorescent reporter dye and a quencher that inhibits dye fluorescence. The intact probe hybridises to its target DNA sequence but is cleaved by DNA polymerase as synthesis of a new DNA strand progresses through the probe target sequence, which results in separation of the reporter dye from its quencher giving rise to a fluorescent signal. To measure MMP-1, TIMP-1, and GAPDH mRNA while excluding detection of genomic DNA, TaqMan systems were developed to span exon junctions. The reporter dyes were 6-carboxyfluorescein for MMP-1 and TIMP-1 and VIC for GAPDH.

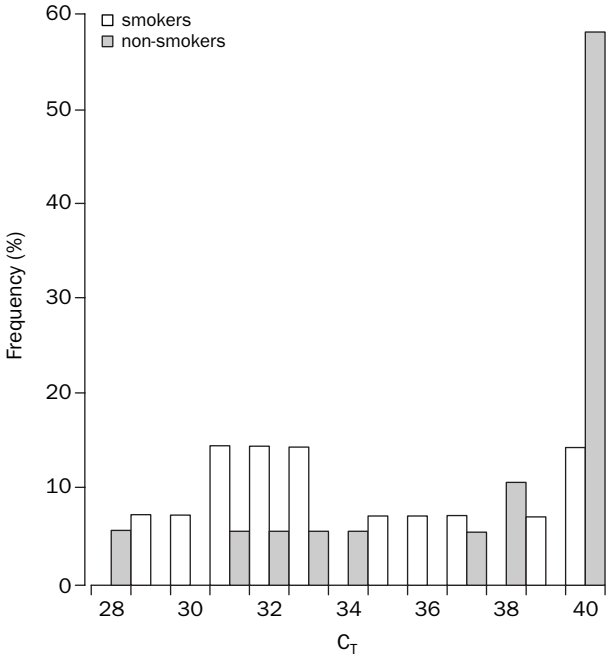
Sequence of PCR primers and sequence specific probes for different targets

that degrade dermal collagen and other extracellular matrix molecules. Collagen is the major extracellular matrix protein in dermis, accounting for at least 70% of its dry weight. The molecular basis of smoking-induced facial ageing is not known but in-vitro studies have shown that tobacco-smoke extract induces MMP-1 and MMP-3 mRNA in skin fibroblasts in vitro.[3] However, tobacco smoke extract has no effect on tissue inhibitor of metalloproteinases 1 (TIMP-1) and TIMP-3 mRNA. TIMPs inhibit the proteolytic activity of MMPs.

We exposed buttock skin to solar-simulating ultraviolet radiation and measured the induction of MMP-1 mRNA in vivo. We noted that in some volunteers there was little or no MMP-1 mRNA in buttock skin before ultraviolet exposure, whereas in others MMP-1 mRNA was readily detectable. We retrospectively asked our volunteers about their smoking status and have compared concentrations of MMP-1 and TIMP-1 mRNA in smokers (three women, 11 men; mean age 29·9 years [SD 7·0]) and non-smokers (nine women, ten men; mean age 27·1 years [6·9]). Smokers generally reported having had about 10–20 cigarettes a day for 3 to 25 years.

Quantitative real-time reverse transcription polymerase chain reaction (TaqMan RT-PCR, PE Applied Biosystems, Foster City, CA, USA) was used to measure mRNA extracted from full thickness 4 mm skin biopsy samples.[4] Analyses were done for MMP-1, TIMP-1, and the housekeeping gene *GAPDH*. The table summarises the technique and gives details of the primers and probes. The PCR cycle at which product detection is significant is known as the threshold cycle (C_T). Thus, the lower the C_T, the higher the concentration of mRNA. A product is reported as absent if it is not detected within 40 cycles. C_T values between 36 and 40 are less reliable for quantitative purposes and cannot be used for relative quantification with reference to a housekeeping gene.

The figure shows the MMP-1 C_T data from non-smokers and smokers. It is clear that the non-smoking data are not normally distributed with 11 (58%) of 19 volunteers having a C_T of 40 compared with 2 (14%) of 14 smokers. Median C_T was higher for non-smokers than smokers (40 *vs* 33·1). We used a two-sample Wilcoxon rank-sum (Mann-Whitney) test to compare the data. MMP-1 was significantly associated with smoking (p=0·013), whereas TIMP-1 and *GAPDH* were not (p=0·433 and p=0·196, respectively). Mean TIMP-1 and *GAPDH* C_T were 21·2 (SD 0·6) and 17·8 (0·66) respectively for non-smokers, and 21·0 (0·69) and 17·6 (0·36) respectively for smokers. Two-sample *t* tests between non-smokers and smokers for TIMP-1 and *GAPDH* showed no differences (p>0·2) confirming the non-parametric Mann-Whitney test results.



MMP-1 mRNA C_T in buttock skin of smokers and non-smokers
The lower the C_T the greater the amount of PCR product.

We have shown that smoking induces MMP-1 mRNA in skin in vivo but has no effect on TIMP-1 mRNA. Furthermore, our findings confirm and validate similar in-vitro results in dermal fibroblasts.[3] A smoking-induced imbalance between MMP-1 and TIMP-1 could be important in the ageing effects of smoking. We did not measure MMP-1 at the protein level but other in-vivo studies have shown a relation between ultraviolet-induced MMP-1 mRNA and protein as well as protein activity.[1] Our volunteers never used sunbeds, thus avoiding any confounding effects of ultraviolet exposure. We suggest that the multiplicative effects of sunlight and smoking on facial ageing[5] occur by induction of MMP-1.

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3 Yin L, Morita A, Tsuji T. Alterations of extracellular matrix induced by tobacco smoke extract. *Arch Dermatol Res* 2000; **292:** 188–94.
4 Brink N, Szamel M, Young AR, Wittern KP, Bergemann J. Comparative quantification of IL-1β, IL-10, IL-10r, TNFα and IL-7 mRNA levels in UV-irradiated human skin in vivo. *Inflamm Res* 2000; **49:** 290–96.
5 Kadunce DP, Burr R, Gress R, Kanner R, Lyon JL, Zone JJ. Cigarette smoking: risk factor for premature facial wrinkling. *Ann Intern Med* 1991; **114:** 840–44.

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