Biochemical and biological effects of air pollution on the function of human skin.

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Background

According to the World Health Organisation, 99% of the world's population live in areas where the air quality exceeds air quality guideline limits, and of even greater concern, over 4.2 million people die annually as a result of exposure to ambient air pollution [1]. Alongside the already established inhalation route, dermal exposure to pollutants has now been implicated in the advancement of skin ageing, as well as a range of skin conditions including psoriasis and atopic dermatitis [2]. This highlights a pressing need for investigation into the mechanisms underlying pollutant-induced skin damage, so appropriate protective strategies can be developed.

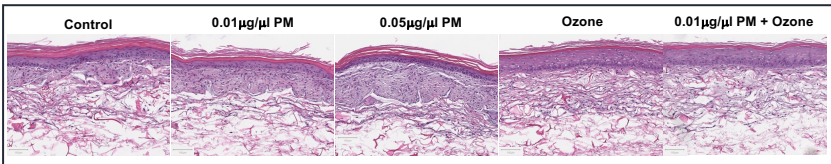
Project Aims

To investigate the biological response to particulate matter (PM) and ozone exposure in human skin equivalents and whether a combination of these pollutants exhibit synergistic effects.

Methods

Phenion full thickness human skin equivalents were exposed to 0.01µg/µl PM, 0.05µg/µl PM, 0.3ppm ozone, or a combination of 0.01µg/µl PM and 0.3ppm ozone for eight hours daily for a total of seven days, before harvesting equivalents. Culture medium was also harvested after 2 and 7 days of exposure. Skin equivalents sections were H&E stained for structural analysis, and the homogenate/medium was used for gene and protein analyses of matrix metalloproteinases (MMP) using qPCR and Western blotting, and prostaglandin-E2 (PGE2) using ELISA assays. Kruskal-Wallis test was performed and corrected for multiple comparisons using Dunn's test for epidermal thickness analysis. One sample t-tests were performed when comparing pollutant groups to the untreated control and unpaired t-tests were performed to assess synergism between the ozone and ozone + 0.01µg/µl PM groups, with * p <0.05, ** p <0.01 and *** p <0.001 representing significance. Results are presented as mean percentage change to the untreated control+SD, with the dashed line representing the untreated control mean. Relative changes in gene expression were evaluated using the $2^{-\Delta\Delta Ct}$ method.

Results



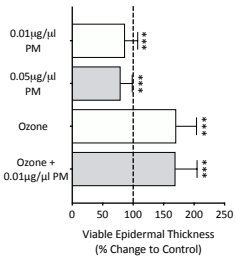


Figure 1: Haematoxylin and eosin staining of skin equivalents after single and combination pollutant exposure for seven days.
Viable epidermal thickness (stratum basale to the stratum granulosum) was measured across the entire skin equivalent to reliably represent pollutant-induced changes. PM seemed to induce a concentration-dependent decrease of 13 and 21% in viable epidermal thickness for 0.01µg/µl PM and 0.05µg/µl PM, respectively, in addition to dysregulation of stratum corneum formation, characteristic of a chloracne phenotype. In contrast, ozone and a combination of ozone and 0.01µg/µl PM induced an increase of around 70% in viable epidermal thickness, most likely as a protective mechanism to limit further damage induced by ozone.

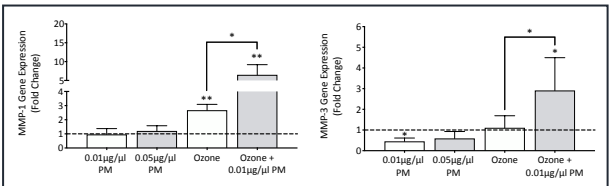


Figure 2: Analysis of MMP gene expression in skin equivalents after single and combination pollutant exposure for seven days.
Skin equivalents were homogenised before extracting RNA and reverse transcribing into cDNA for qPCR gene expression analysis. PM induced no changes in MMP-1 gene expression, however did induce a significant decrease in MMP-3 gene expression. Ozone and combination pollution induced a significant increase in MMP-1 gene expression, and combination pollution induced an increase in MMP-3 gene expression. There were also synergistic effects observed after combination pollution exposure for both MMP-1 and MMP-3 expression.

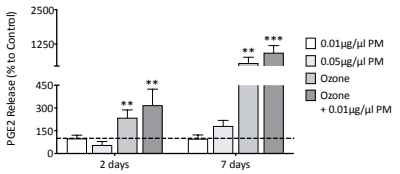


Figure 3: Analysis of PGE2 release in conditioned medium from skin equivalents after single and combination pollutant exposure for seven days.
Medium was removed from skin equivalents after 2 & 7 days of pollution exposure and used for analysis of PGE2 secretion using ELISA. PM exposure induced no changes in PGE2 secretion, however ozone and combination pollution induced significant increases.

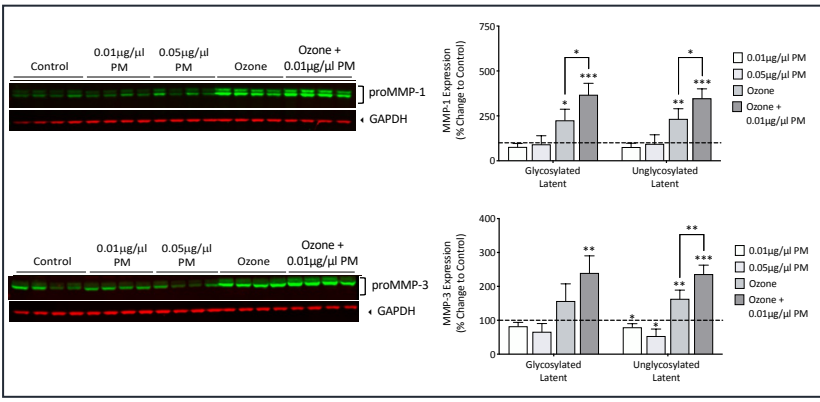


Figure 4: Analysis of MMP protein expression in skin equivalents after single and combination pollutant exposure for seven days.
Skin equivalents were homogenised and the protein extracted for Western blotting analysis of MMP-1 and MMP-3 protein expression. Glycosylated and unglycosylated MMPs were normalised to GAPDH using densitometric analysis. Similar to gene expression, PM induced no changes in MMP-1 protein expression, however decreases in MMP-3 expression. In contrast ozone and combination pollution induced a significant increase in both MMP-1 and MMP-3 protein expression. There were also synergistic effects observed after combination pollution exposure in both MMP-1 and MMP-3 expression.

Conclusion

This study showed that PM is only capable of surface level damage, whereas ozone is capable of causing downstream damage, inducing increases in MMP-1, MMP-3 gene/protein expression and PGE2 secretion, indicating the acceleration of the skin ageing phenotype. Synergistic effects were also observed, indicating that although PM does not cause major damage alone, when combined with ozone it shows the potential to augment ozone-induced skin damage.

References

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