OZONE EFFECT ON RAGWEED POLLEN VIABILITY AND NAD(P)H OXIDASE ACTIVITY

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Background

The increase in respiratory diseases arising from allergies in industrialised countries in recent years is considered to be linked to changes in certain environmental factors. One such factor relates to the higher levels of atmospheric pollution and the greater presence and distribution of allergenic taxa. We investigated the effects of ozone (O₃), the main component of the photochemical smog, by exposing ragweed (Ambrosia artemisiifolia) pollen to a high O₃ concentration (100 nL L⁻¹) for 7 days. We specifically evaluated: pollen reactive oxygen species (ROS) and nitric oxide (NO) content, as NO is considered to be a mediator of inflammatory responses; activity of the nicotinamide adenine dinucleotide phosphate (NAD[P]H) oxidase, which can generate ROS; expression of the major ragweed pollen allergens; and pollen viability.

Results

We investigated the impact of O₃ on ROS and allergen content of ragweed pollen. Pollen was exposed to acute O₃ fumigation, with analysis of pollen viability, ROS and NO content, activity of NAD(P)H oxidase, and expression of major allergens. There was decreased pollen viability after O₃ fumigation, which indicates damage to the pollen membrane system, although the ROS and NO contents were not changed or were only slightly induced, respectively. Ozone exposure induced a significant enhancement of the ROS-generating enzyme NAD(P)H oxidase. The major allergenic proteins released from ragweed pollen ranged from 8 kDa to 43 kDa, with similar protein pattern profiles in both control and O₃-fumigated pollen. The major antigenic component of ragweed pollen, Amb a 1, was identified as a band visible at about 38 kDa, and Western blotting analysis revealed that its content did not change after O₃ exposure. We also examined the expression profiles of the major ragweed pollen allergens: Amb a 1 and Amb a 2, which are proteins belonging to the pectate lyase family, and profilin 1 and profilin 2, which are proteins involved in signal transduction from the outer cell membrane to the inside of the cell, and in the regulation of actin polymerisation. Our data show that O₃ exposure did not affect the expression of the major ragweed pollen allergens.

Conclusions

Pollen represents a critical stage in the life cycle of plants, as viable pollen is crucial for efficient sexual reproduction in plants. Our data indicate that exposition of ragweed pollen to realistic O₃ concentrations reduces pollen viability. As the pollen viability is significantly related to pollen germination and the length of the pollen tubes, effects on the reproduction of ragweed in polluted situations should be taken into account. The present study also indicates that there is an impact of the air pollutant O₃ on the ROS/NO content, NAD(P)H oxidase activity and allergens of ragweed pollen grains. While the intrinsic ROS and allergen content were not affected by O₃ fumigation, there was significant enhancement of the activity of the ROS-generating enzyme NAD(P)H oxidase. This enzyme was almost completely released after short times of in-vitro pollen
hydration, as occurs in nature when pollen comes into contact with the cells of the respiratory apparatus. We conclude that realistic doses of O₃ can increase ragweed pollen allergenicity through stimulation of NAD(P)H-oxidase-mediated ROS generation at the airways level.