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A modified Ames assay reveals the mutagenicity of native cigarette mainstream smoke and its gas vapour phase

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Abstract

The evaluation of the mutagenic activity of cigarette smoke is mostly based on studies with condensates or extracts in the standard Ames assay. These samples only insufficiently reflect the composition of the actual generated aerosol. Therefore, such atmospheres should be analysed in their native composition to gain a real signal of its mutagenic capacity. Based on the technical difficulties of testing native air contaminants, there are no accepted methods for effective exposure of bacteria under such conditions. Therefore, we established a new experimental approach for direct exposure of bacteria in a modified CULTEX® system (Patent no. DE 19801763/PCT/EP99/00295) connected to a smoking machine. This allowed us to investigate the mutagenic activity of native mainstream smoke of the research cigarette K2R4F by exposure of *Salmonella* Typhimurium strains. In comparison to studies using the plate incorporation assay, the direct exposure of bacteria to smoke on the agar surface enhances contact to the aerosols. By using this modification of the Ames assay, we demonstrate that it is possible to analyse the effects of native whole smoke and the gas vapour phase of cigarettes directly and achieve a dose-dependent induction of revertants. In a number of experiments, the treatment of strains TA98 and TA100 with whole smoke and the gas vapour phase of K2R4F cigarettes resulted in the induction of revertants dependent on the dilution of smoke and the number of cigarettes smoked. Our alternative procedure of exposing bacteria directly to gases and complex mixtures offers new possibilities in the field of inhalation genotoxicology for the evaluation of genotoxicity in the Ames assay.
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Keywords: Ames assay; Complex mixtures; Native cigarette smoke; Direct exposure; CULTEX® system

Introduction

Previous studies on the toxic and mutagenic potency of complex gas mixtures such as urban air, welding fumes and cigarette smoke have focused on the particulate fraction, which can be collected on filters, fractionated, analysed and assayed using standard chemical methods (Kier et al., 1974; IARC, 1986; Roemer et al., 2002; Andreoli et al., 2003; Foy et al., 2004). The Ames assay (Ames et al., 1975; Maron and Ames, 1983) has been widely used to demonstrate the mutagenic potential of cigarette smoke condensate (CSC) and the gas vapour phase (GVP) which has been collected by bubbling through phosphate-buffered saline (PBS). However, these samples represent only artificial

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fractions of the actual smoke, and interactions between the particles, gases and volatile compounds are not taken into consideration. Only a few attempts have been made (Montieth et al., 1986; Balansky et al., 1987) to demonstrate the mutagenic effect of native cigarette smoke. In these studies, the induction of revertants in the Ames assay was shown by exposing bacteria of different *Salmonella* Typhimurium strains within plastic boxes filled with cigarette smoke or to a continuous horizontal flow of the smoke above the cultures. In general, the results demonstrated mutagenic effects of the smoke, but without a dose–response relationship. For proper analysis of complex atmospheres, both a sensitive bioassay and an appropriate exposure device have to be used.

To achieve such conditions, the CULTEX®-system, which has been successfully used for demonstrating cytotoxicity of different cigarette brands (Aufderheide et al., 2002; Aufderheide et al., 2003; Fukano et al., 2004) was modified to expose bacteria in the Ames assay, (CULTEX®-B, CULTEX Laboratories, Germany), (Aufderheide and Mohr, 2004). Strains TA98 and TA100 are sensitive to CSC, therefore both strains are widely used tester strains to reveal the mutagenic capacity of CSC (Asita et al., 1991; Balansky et al., 1987; Steele et al., 1995).

In this study, we analysed the mutagenic activity of native cigarette whole smoke and its GVP. The combination of a smoking machine especially designed for *in vitro* work, a modified CULTEX®-B module and a modified Ames assay allowed us to demonstrate a dose-dependent induction of revertants after exposing the bacteria of strains TA98 and TA100 to native cigarette smoke.

Materials and methods

Smoke generation

A varying number of K2R4F cigarettes (University of Kentucky Tobacco and Health Research Institute in Lexington, Kentucky, USA) were smoked according to ISO 3308 guidelines (35 ml puff volume, 2 s duration, 1 puff/min). The GVP was generated by trapping the particulate matter on Cambridge filters that were placed between the smoking machine and the dilution system. The freshly generated smoke was sucked via the dynamic dilution device through the exposure modules controlled by mass flow controllers and valves. Smoke puffs were transported into the dynamic dilution system and immediately mixed with a constant flow of synthetic air (0.5, 1.0 and 1.5 l/min). In this way, the discontinuously generated smoke was diluted and converted into a continuous aerosol flow, which was sucked via negative pressure from a vacuum pump at different flow rates (8, 20, 50 and 100 ml/min) through the CULTEX®-B module (CULTEX Laboratories, Germany). The flow rates for each position within the module were controlled separately by needle valves to guarantee a homogeneous gas stream and particle deposition over the exposure positions. During the intervals of puff generation the bacteria were exposed to synthetic air.

Particle deposition

To determine the particle deposition on the bacterial plates, Cambridge filters were placed on the top of plates with a dummy metal plate of the same height as the agar within the exposure module. Total particle mass flow was measured for each of the three exposure positions. One to three K2R4F cigarettes were smoked at dilutions 0.5, 1.0 and 1.5 l/min flow of synthetic air at flow rates of 8, 20, 50 and 100 ml/min through the exposure module. The Cambridge filters were extracted in methanol by shaking on an orbital shaker for 30 min. The fluorescence of the extract was measured (excitation 355 nm, emission 485 nm). The tar concentration was calculated by comparison to a standard. The deposition or recovery rate was calculated as a percentage of the total tar mass.

Bacterial strains and culture conditions

The tester strains TA98 and TA100 were originally obtained from the laboratory of Bruce Ames (University of California, Berkeley, USA). Single colonies were isolated and strain characteristics were checked according to Maron and Ames (1983). A frozen bacterial stock suspension was grown overnight at 37 °C in a shaking incubator in 20 ml nutrient broth. Bacteria were in late log phase when used. To calculate the bacterial number before seeding them onto the agar, aliquots of the bacterial suspension were counted using an automated cell counter and analyser system (CASY Modell TT, Schärfe System GmbH, Germany). The bacteria were seeded in Petri dishes of minimised scale (35 mm in diameter, Nunc #150318) with and without overlay agar (spread culture).

Mutagenicity assay

The overlay agar Method was consistent with the method published by Maron and Ames (1983). Using the spread culture method the bacteria were poured directly onto the selective agar plates (composition: 1.5% agar, 2% glucose, Vogel /Bonner medium) already complemented with a histidine/biotin solution (0.5 mM).

Concurrent negative and positive controls (according to OECD guideline 471) were performed in all

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