Impact of test conditions on in vitro cytotoxicity measurements of mainstream cigarette smoke using a whole smoke exposure system

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Abstract

The purpose of this study was to evaluate the impact of several factors on smoke cytotoxicity measurements in an in vitro whole smoke exposure system. Effects of incubation time after smoke exposure, cell types used, and smoke regimens on the cytotoxicity of cigarette smoke were investigated. Mainstream cigarette smoke was generated from the 3R4F reference cigarettes using a VC10 smoking robot under the ISO regimen (35/602 without blocking of filter ventilation) or the HCl regimen (55/302 with complete blocking of filter ventilation). Cells were exposed to fresh whole smoke (WS) in the VITROCELL® system, and cytotoxicity was evaluated using the neutral red uptake (NRU) assay. Results showed 24 hrs after smoke exposure appeared to be an optimal time-point to assess smoke cytotoxicity. CHO cells were more sensitive to smoke-induced cytotoxic effects than A549 cells. Smoke regimen evaluation was conducted in both cigarette smoke condensate (CSC) and WS. For ISC testing, the cytotoxicity decreased going from the ISO regimen to HCl regimen on a per unit of total particulate matter (TPM) basis. For WS exposure testing, cytotoxicity under the ISO regimen was less than that under the HCl regimen when smoke doses were expressed as % of cigarette smoke; notably, when smoke doses were converted to TPM (µg), cytotoxicity under the HCl regimen was less than that under the ISO regimen. A clear dose-response relationship between cell viability and smoke doses was observed under all test conditions. These data indicated that this in vitro smoke exposure system can be a useful tool to study the toxicological effects of WS, and the test conditions can have an important impact on the results of cytotoxicity evaluation of cigarette smoke.

Introduction

With the advance of in vitro whole smoke (WS) exposure technology, more studies now evaluate the fresh WS for toxicological responses (Aulderheide and Gressmann, 2008; Okuwa et al., 2010). WS might reflect completely the biological effects of native smoke. Cytotoxicity assay is a part of a battery of in vitro assays for assessing the potential toxicity of tobacco products. Here we evaluated the impact of test conditions on smoke cytotoxicity measurements in an in vitro whole smoke exposure system.

Materials and Methods

Cigarettes

3R4F reference cigarettes (Lexington, Kentucky, USA) were conditioned at 22 ± 1 °C and 60 ± 3% relative humidity for at least 48 hrs before being smoked.

Cell culture

Chinese hamster ovary (CHO) cells and human lung adenocarcinoma epithelial cell line (A549) were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Approximately 24 hrs before exposure, cells were trypsinized and seeded onto 96 well plates (Corning, NY, USA) at a density of 1 × 104 cells/well for cigarette smoke condensate (CSC) treatment, or seeded onto insert membranes (diameter size 0.4 µm, growth area 1.12 cm²) (Corning) at a density of 3.5 × 104 cells/well for WS exposure system.

Cigarette smoke generation and exposure

WS exposure was performed on the VITROCELL® system (VITROCEL, Waldkirch, Germany). Under ISO regimen (35/602 without blocking of filter ventilation), the dilution air flow rates were 0.75, 2.25 or 3.75 L/min for 4 smoke concentrations. Under HCl regimen (55/302 with complete blocking of filter ventilation), the dilution air flow rates were 0.1, 1.2, 3.55 or 5.9 L/min. As a negative control, cells were exposed to clean air at a flow rate of 5.95 min. Smoke doses were expressed as % of cigarette smoke (% of CS) (Okuwa et al., 2010). CSC collections were done with a Borgwaldt RM-20H smoking machine (Borgwaldt RC, Hamburg, Germany). CSC and WS regimens were treated with CSC solutions at the following concentrations for 24 hrs: 1, 10, 50, 75, 100, 120, 140, 160, and 200 µg/mL.

NRU cytotoxicity assay

The neutral red uptake (NRU) cytotoxicity assay for smoke was performed according to the method recommended by Health Canada (Health Canada Official Method T-602, 2004) with some modifications. The absorbance was measured at 540 nm with a microplate reader (Model 680, Bio-Rad Laboratories, Tokyo, Japan). EC50 values were estimated using the Origin 8.0 software.

Statistical analysis

The data obtained from in vitro cytotoxicity assay was expressed as Mean ± SD. The differences in the NRU values between smoke under different test conditions were analyzed by a two tailed t-test as well as the difference in EC50 values under different smoking regimens. A P value of <0.05 was considered as statistically significant.

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Conclusions

• A clear dose-response relationship between cell viability and smoke doses was observed under all test conditions.
• 24 hrs after smoke exposure appeared to be an optimal time-point to assess smoke cytotoxicity.
• CHO cells were more sensitive to smoke-induced cytotoxic effects than A549 cells.
• For ISC testing, the cytotoxicity decreased going from the ISO regimen to HCl regimen on a per unit of total particulate matter (TPM) basis.
• For WS exposure testing, cytotoxicity under the ISO regimen was less than that under the HCl regimen when smoke doses were expressed as % of cigarette smoke; notably, when smoke doses were converted to TPM (µg), cytotoxicity under the HCl regimen was less than that under the ISO regimen.

References


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