Determination Of The Cytotoxicity Of Whole Cigarette Smoke

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INTRODUCTION

Cigarette smoke is a complex mixture of thousands of chemicals distributed between two phases, particulate (4-9%) and vapour phase (96-91%), which together are termed 'whole smoke'[1-2] (Fig. 1)

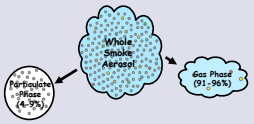


Fig.1: Breakdown of smoke constituents

Historically, the *in vitro* toxicological assessment of smoke has measured the activity of the particulate phase, following collection on filter pads. However, vapour phase contains components such as aldehydes and oxides of nitrogen which may induce biological effects.

This poster describes a method of exposing NCI-H292 human lung epithelial cells to 'whole mainstream cigarette smoke' at the air-liquid interface.

OBJECTIVE: To compare the cytotoxicity of 'whole smoke' and 'smoke vapour phase' to cells maintained at the air liquid interface.

METHODS AND MATERIALS

Smoke Generation and Dilution

Two Virginia tobacco based filtered cigarettes were examined:

- Cigarette A 0% filter ventilation, 17mg particulate delivery.
- Cigarette B 70% filter ventilation, 6mg particulate delivery.

A RM20s smoking engine (Borgwaldt Technik GmbH) was used to generate and dilute mainstream smoke with filtered air from 1/2 to 1/700,000 (v/v, smoke/air). The RM20s delivered diluted whole smoke to NCI-H292 lung epithelial cells for 30 min. The cells were maintained at the air liquid interface in an exposure chamber, and provided with a continuous supply of fresh Dulbecco's modified Eagle's medium 37ºC (Fig. 2).

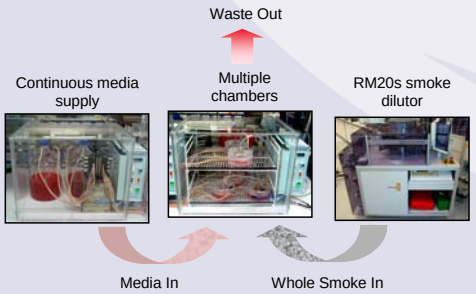


Fig. 2 : Whole Smoke Exposure System

Smoke Exposure

Confluent cultures of NCI-H292 human lung epithelial cells on semi-permeable membranes (Transwell™, Corning) were placed in a sterile, BAT-designed perspex exposure chamber (patent pending - WO 03/100417 A1) which allows cells to be exposed to whole smoke/vapour phase at the air-liquid interface. (Figs. 3 & 4)

3 critical chamber design characteristics:

- A self-levelling media device.
- An island to distribute media and ensure complete media exchange.
- A smoke distribution plate that distributes smoke homogenously.

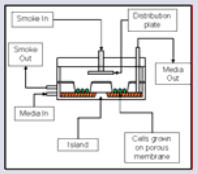


Fig. 3: Schematic Of Exposure Chamber

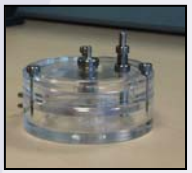


Fig. 4: Exposure Chamber

Smoke Characterisation

The smoke particulate phase was monitored using two methods:

- A condensation particle counter measured the concentration of particles in the exposure chamber.
- Smoke particulate matter deposited on the Transwells was quantified by analysing total fluorescence.

Decreases in smoke dilution resulted in increases in particle concentration (Fig. 5) and more particles deposited on the Transwells (Fig. 6).

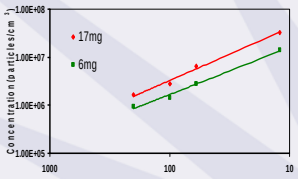
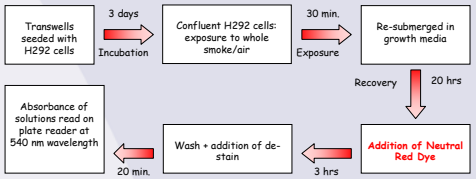


Fig. 5: Particle concentration of test atmosphere

Fig. 6: Measurement of deposited particulate matter

Determination Of Cytotoxicity

The flow diagram describes the protocol used to determine the cytotoxicity of whole smoke using the Neutral Red Cytotoxicity Assay:



The results from smoke exposure were expressed as a percentage of control H292 cells exposed to filtered air under the same conditions.

RESULTS

Whole Smoke

For each cigarette, eight serial dilutions over the following dose range were used:

- Cig A (non-ventilated, 17mg) = 1/20 to 1/500
- Cig B (70% ventilated, 6mg)= 1/1.5 to 1/200

Each experiment was repeated on 3 separate occasions. The reduction in H292 cell viability from the exposure to whole smoke from cigarettes A & B can be seen in Fig. 7.

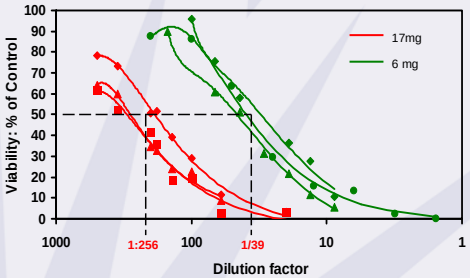


Fig. 7: 17mg vs. 6mg

Whole mainstream smoke, from both test cigarettes, reduced cell viability in a dose-dependent manner. The dilutions (mean ± SD) giving a 50% reduction in cell viability were calculated for each cigarette type and demonstrated a mean reduction of 84.7% in cytotoxicity for the lower 6mg delivery cigarette (p=0.026)

% reduction cytotoxicity = (256-39)/256 X 100

= 84.7%

Vapour Phase

The contribution from the smoke vapour phase and smoke particulate to the cytotoxicity was determined by trapping smoke particulate matter on a Cambridge filter pad before it entered the exposure chamber. (Fig. 8). Fig. 9 shows the reduction in cytotoxicity as a result of removing the particulate phase of smoke.

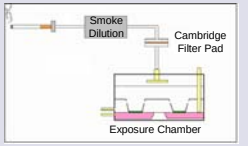


Fig. 8: Removal of particulate matter from whole smoke

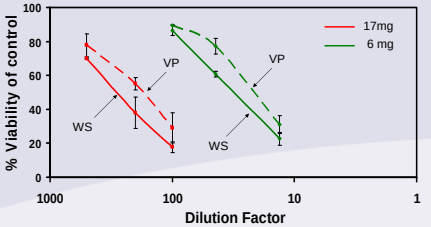


Fig. 9: Whole smoke (WS) vs. Vapour phase (VP)

A differential response between whole smoke and vapour phase was observed. The response of vapour phase was significantly less (~20%) than that of whole smoke (Wilcoxon non-parametric test for paired data sets p=0.005)

DISCUSSION.

- The results from exposure to whole smoke (Fig. 7) showed a dose dependent decrease in cell viability with decreases in smoke dilution.
- The cytotoxicity of the smoke from the lower delivery cigarette B (6 mg) was significantly lower than that of the higher delivery cigarette A (17 mg). The comparative inhibitory dilutions for the cigarettes used in this study were of the same rank order as the predicted delivery of compounds in mainstream smoke.
- Exposure of cells to smoke vapour phase only, demonstrated that ~80% of the cytotoxic effect was from vapour phase components and ~20% from the particulate phase.
- In conclusion, we have developed a novel system suitable for *in vitro* exposure to mainstream cigarette smoke for potential toxicological assessments. We propose that this system may give complimentary information concerning the toxicity of smoke in addition to experiments using cigarette smoke particulate matter.

References

1) Baker, R. R.(1999) In: Tobacco, production, chemistry and technology, Eds Layten Davis, D. and Nielson, Mark T. Blackwell Science. Chapter 12: Smoke Chemistry pp 398-439.

2) Keith, C.H. and Tesh, P.G. (1965) Measurement of the total smoke issuing from a burning cigarette, Tobacco Science. Vol 9, pp61-64.