MENTHOLATED AND NON-MENTHOLATED CIGARETTES ALTER TRANSEPITHELIAL ELECTRICAL RESISTANCE OF CALU-3 HUMAN BRONCHIAL EPITHELIAL CELLS

Student Researcher: Jorjana Alakayak, Mt. Edgecumbe High School
Mentor: Cindy Knall, PhD, WWAMI Biomedical Program, University of Alaska Anchorage

A quarter of all smokers in the US smoke mentholated cigarettes with the frequency of use especially high among African Americans. Although African Americans smoke fewer cigarettes per day, they have a higher incidence of smoking related diseases. The reason for this is unknown. Tight junctions between lung epithelial cells are structures that act as a blockade against inhaled foreign particles. Lung cells from smokers exposed to cigarette smoke show an alteration in tight junction function as measured by reduced electrical resistance of the epithelial monolayer. This allows foreign matter a greater chance of passing through the lung barrier. We hypothesized that the smoke from mentholated cigarettes would have a greater effect on lung epithelial cells compared to non-mentholated cigarettes.

To test this hypothesis, we exposed Calu-3 human bronchial epithelial cells to cigarette smoke using an *in vitro* air-liquid interface exposure system. Smoke from either mentholated or non-mentholated cigarettes was generated using the Federal Trade Commission smoking profile. After exposing Calu-3 cells to smoke from either mentholated or non-mentholated cigarettes, transepithelial electrical resistance (TER) was measured to determine the effect menthol has on the tight junctions of the Calu-3 cells. Although smoke from mentholated cigarettes caused a significant decrease in TER compared to air alone, the decrease in TER was not significantly different from that seen with non-mentholated cigarettes. Therefore, the data suggest that any increased risk from mentholated cigarettes is not due to a greater effect on TER compared to non-mentholated cigarettes.

BACKGROUND

The major cause of chronic obstructive pulmonary disease and lung cancer is cigarette smoking. In the United States almost 500,000 deaths per year are linked to cigarette smoking. There are over 4,000 chemicals in cigarette smoke. Mentholated cigarettes[1] contain menthol which is extracted from peppermint plants.[2,3] Menthol acts as a coolant and a mild local anesthetic. Consequently, it may allow smokers to breathe more deeply when taking a puff thus exposing them to more nicotine and carcinogens.

Tight junctions are the structures that are the foundation for epithelial blockades acting as guards to prevent particles and fluids from flowing freely.[4] Tight junction integrity is lost when mainstream cigarette smoke reaches the lung epithelium. Exposure to smoke affects the cells which react by loosening their barrier-like structure.[5] This causes a decrease in the TER of the epithelial cell monolayer. The effect that smoke from mentholated cigarettes has on tight junctions is unknown.

METHODS

Calu-3 human bronchial epithelial cells were grown in Delbecco's Modified Eagles Media containing fetal bovine serum, L-glutamine, and penicillin/streptomycin and maintained at 37°C and 5% CO_2 during growth. Cells were plated into Costar Transwells (Corning, Acton, Mass) and grown to 100% confluence. Cells were changed into exposure media 90 minutes before the exposure. The exposure media contains HEPES, which helps maintain a balance in the cell's pH while the cells are exposed in fresh room air or smoke containing room air.

Cells were exposed to mainstream cigarette smoke using an *in vitro* air-liquid interface exposure system (Figure 1). This system is composed of an InExpose smoke generator (SciReq, Montreal, Quebec), VITROCELL 12 chambers (VITROCELL, Waldkirch, Germany) and Mass-Trak flow controllers (Sierra Instruments, Monterey, Calif). The smoke was generated from Newport Full Flavor (mentholated cigarette) (Lorillard, Greensboro, NC) or Marlboro Full Flavor (non-mentholated cigarette) (Philip Morris, Richmond, Va) using the Federal Trade Commission smoking profile (35 mL puff volume, 2 second puff, every 60 seconds to a cigarette butt length of 35 mm). Smoke particulate matter was collected from individual cigarettes of each type using Whatman EPM 2000 High-Volume Air Sampling Filters (Whatman, Brentford, United Kingdom).

Measurements of TER were made using a Millicell-ERS (Millipore, Billerica, Mass) before the exposure, immediately after and at 15, 30, 45, and

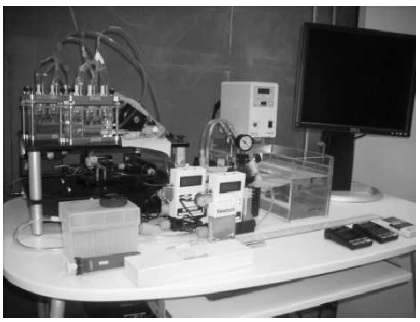

Fig 1. In vitro Air-liquid Interface Exposure System

Fig 2. Mean coloration of smoke exposed filters. Mean coloration was determined by color sampling individual filters and determining the average value for red, green and blue coloration from 3 filters for each cigarette type

60 minutes after exposure to the cigarette smoke. Data were analyzed and graphs generated using Excel (Microsoft, Redmond, Wash) and KaleidaGraph (Synergy Software, Reading, Pa). TER values were normalized to the TER values taken before the exposure and means were calculated from 3 replicates per experiment and 3 independent experiments. Similarities and differences among groups were tested using two-way ANOVA with repeated measures.

RESULTS

The experiments successfully generated data addressing the effects on tight junction function of mentholated and non-mentholated cigarette smoke. The results of particle collection on the air sampling filters exposed to smoke from either Newport Full Flavor or Marlboro Full Flavor suggest that Newport cigarettes generated more particulate matter than the non-mentholated Marlboro cigarettes (Figure 2). Calu-3 cells exposed to either mentholated ($P<0.5$) or non-mentholated ($P<0.0003$) smoke showed a significant decrease in electrical resistance compared to air exposed cells (Figure 3). The difference in TER between cells exposed to mentholated or non-mentholated smoke was not significant (Figure 3).

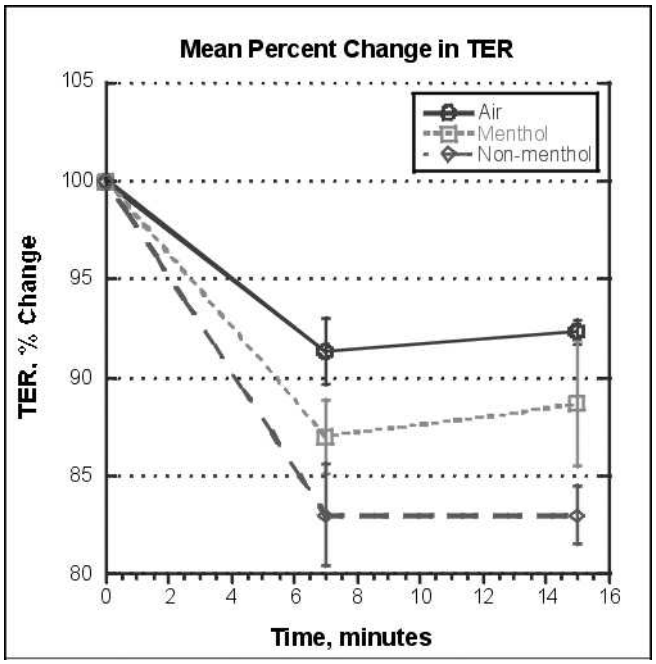

Fig 3. Effect of Smoke on TER. Data shown are the mean ± SEM of the percent change in TER for each condition from 3 independent experiments with 3 replicates each

CONCLUSION

The initial hypothesis was proven incorrect as the smoke from mentholated cigarettes was no more harmful than smoke from non-mentholated cigarettes. Although mentholated cigarettes are advertised as less irritating, our data showed that the smoke from these cigarettes irritates lung cells and causes them to lose tight junction function. This may be due to the mentholated cigarettes producing more particulate matter than the non-mentholated cigarettes.

REFERENCES

1. O'Connor RJ. What brands are US smokers under 25 choosing? *Tob Control*. 2005;14:213–215.
2. Clark PI, Gardiner PS, Djordjevic MV, Leischow SJ, Robinson RG. Menthol cigarettes: setting the research agenda. *Nicotine & Tobacco Research*. 2004;6(S1):S5–S9.
3. WiseGeek. What is Menthol? Available at http://www.wisegeek.com/what-is-menthol.htm. Accessed August 6, 2007.
4. Schneeberger EE, Lynch RD. The tight junction: a multifunctional complex. *Am J Physiol Cell Physiol*. 2004;286:C1213–C1228.
5. Olivera DS, Boggs SE, Beenhouwer C, Aden J, Knall C. Cellular mechanisms of mainstream cigarette smoke-induced lung epithelial tight junction permeability changes in vitro. *Inhal Toxicol*. 2007;19:13–22.