Toxicity of inhaled Nicotine and Pyruvic Acid Aerosols (separately and combined) in Sprague-Dawley Rats in a 28-day OECD 412 Inhalation Study

Introduction

Cigarette smoking can cause severe diseases such as cardiovascular disease, lung cancer, and chronic obstructive pulmonary disease. Health concerns are caused primarily by smoke constituents other than nicotine. Therefore, provision of nicotine during nicotine replacement therapy (NRTs) or by using electronic nicotine delivery systems (“e-cigarettes”) may be considered a less harmful way to facilitate smoking cessation or to reduce harm by smoking fewer tobacco cigarettes (Benowitz and Goniewicz, 2013, Franck et al., 2014). Nicotine administration via inhalation is an attractive approach for a NRT because it can deliver nicotine rapidly to the brain, thus better mimicking the effects of discontinuous/episodic exposure achieved if smoking cigarettes. Aerosols of nicotine pyruvate have been proposed as improved forms of NRT for those who are not willing to stop smoking. Such aerosols have favorable pharmacodynamic properties and subjective responses (Rose et al., 2010) but the toxicity of repeated or extended use of aerosols of nicotine pyruvate has not been investigated.

Here we report on a comprehensive repeated-dose inhalation toxicity study on aerosols of nicotine and pyruvic acid aerosols (separately and combined) in Sprague-Dawley rats in a 28-day OECD 412 (OECD TG 412, OECD, 2009) with focus on systemic toxicity and respiratory endpoints. In addition, molecular endpoints (transcriptomics and lipidomics) were assessed in an separate cohort of rats providing mechanistic insights on the classical toxicological endpoints (published elsewhere).

Systemic effects (cont’d)

Liver effects: nicotine concentration-dependent

- Cytoplasmic vacuolation
- Vacuoles contains PAS-positive material, likely to be lipids

Clinical Chemistry: nicotine concentration-dependent changes in:

- Alkaline phosphatase and alanine aminotransferase - higher
- Total cholesterol and glucose - lower

Bio-monitoring

Nicotine form nebulized aerosol is taken up as evidenced by the amount of 5 nicotine metabolites recovered in 24-h urine:

- \( ^{13}C_{15} \) nicotine: 0.308 mg/kg, \( ^{13}C_{14} \) nicotine: 0.308 mg/kg, nicotine pyruvate: 33.9 mg/kg, nicotine + nicotine pyruvate (LOW): 0.111 mg/kg, nicotine + nicotine pyruvate (HIGH): 0.154 mg/kg

Test atmosphere characterization

Target concentrations were met

- Particulate (droplet) size: equally respirable aerosols
  - MMAD: 1.4 - 2.0 µm, GSD: 1.7 – 2.2

Exposure set-up

- Test atmosphere monitoring: nicotine, pyruvate, T, RH, droplet size distribution (MMAD, GSD)
- Bio-monitoring: respiratory physiology, urinary nicotine metabolites (24 hour urine)
- Systemic end points during study: clinical observations, body weight, food and water consumption
- Systemic end points at the end of the study: hematology, clinical chemistry, pulmonary inflammation (cells in broncho-alveolar lavage fluid, BALF)
- Histopathology of the respiratory tract, non-respiratory tract organs

Summary and Conclusion

- The nebulizer approach was successful for generating nicotine-containing aerosols with particle (droplet) sizes similar to those from cigarette smoke in experimental setups
- All aerosols were inhaled efficiently and were well tolerated by the rats in the present 28-day inhalation study
- Systemic effects indicated low levels of toxicity for nicotine-containing aerosols in the presence or absence of pyruvic acid. Changes included slight hematological changes and reduction of liver enzymes
- Mild irritation of the larynx was observed in the nicotine-containing aerosol, while the control groups revealed very mild changes probably due to particle irritation
- Exposure to nicotine-aerosols causes an increased vacuolation of hepatic tissue, vacuoles contained glycogen
- In conclusion, these results suggest that exposure to high concentrations of nicotine (alone or in combination with pyruvic acid) did not result in substantial toxicity and further suggest an alteration in metabolism, as seen in the liver

References


Histopathology – Respiratory tract

Minimal to mild epithelia changes in nicotine-exposed rats
- Nose: low level hyperplasia of the respiratory epithelium, nose level 1
- Larynx: minimal to mild hyperplasia of squamous epithelium, minimal aqueous metaplasia, increased epithelia thickness at vocal cords
- Lung: no noteworthy findings, no obvious lung inflammation

Histopathology – Non-respiratory tract organs

Liver effects: nicotine concentration-dependent
- Complimentary vacuolation,
- Vacuoles contain PHA-positive material, likely to be consist of glycogen granules

Systemic effects

Reduced body weight gain in nicotine treated male rats

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