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The regional hydrolysis of ethyl acrylate to acrylic acid in the rat nasal cavity

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SUMMARY

Cytotoxicity is primarily limited to the olfactory epithelium of the dorsal meatus region of the nasal cavity of rodents following inhalation exposure to acrylic monomers. To investigate the biochemical basis for this effect, three regions of the Fischer F344N rat nasal cavity were evaluated for carboxylesterase activity for the representative acrylic ester, ethyl acrylate. Prior studies have indicated that the rodent olfactory epithelium is sensitive to the cytotoxic effects of short chain organic acids. In this study, no regional difference in carboxylesterase activity was observed between sensitive and non-sensitive regions of olfactory epithelium. Respiratory epithelium (resistant to cytotoxicity) was found to be have a much lower rate of carboxylesterase activity than olfactory epithelium. These results suggest that the regional distribution of cytotoxicity observed in the rat nasal cavity at high concentrations of inhaled acrylic monomers may be due in part to the amount of released organic acid following deposition. However, the observation of the same esterase activity in sensitive and nonsensitive olfactory regions suggests that nasal air flow patterns and regional deposition may also be critical factors.

INTRODUCTION

Esters of acrylic acid are used in the synthesis of aqueous polymers that form the raw materials for the production of a wide variety of coatings and adhesives. Methyl, ethyl, and butyl acrylate vapors have been evaluated in chronic inhalation studies [1,2]. No treatment-related increases in tumors were observed in these studies. However, a marked regional cytotoxicity was observed in the nasal cavity of both rats and mice at high exposure levels of the monomers. Specifically, cytotoxicity was preferen-

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tially induced in the olfactory epithelium in the dorsal meatus region, and secondarily on the medial processes of olfactory epithelium of the lateral turbinates and the anterior regions of the ethmoid turbinates. Remarkably, toxicity was not observed in the respiratory epithelium, although this tissue is sensitive to the toxic effects of other volatile organic compounds (reviewed in Ref. 3). Furthermore, no signs of toxicity were observed in the lung or other internal tissues. The same olfactory epithelial toxicity in the dorsal meatus region of the nasal cavity was observed following a subchronic study with acrylic acid (AA), the parent acid for the acrylic esters [4]. In each case, cytoxicity in the sensitive olfactory epithelium was followed by replacement of the olfactory tissue with resistant respiratory tissue, a process that has been described as 'respiratory metaplasia' [1]. No progression of acrylate-induced respiratory metaplasia to neoplasia has been observed in chronic studies with rats and mice [1,2].

Studies with other volatile organic acids and esters (notably, a class of solvents called the 'dibasic esters', a mixture of methyl esters of adipic, glutaric, and succinic acids) have resulted in a similar regional distribution of cytotoxic effects [5,6]. Biochemical studies have suggested that the organic acids released upon hydrolysis of the esters in the nasal cavity mediate the toxicity to the olfactory epithelium [7–10]. Since the rodent nasal cavity has a high concentration of carboxylesterase activity [11–13], and studies with homogenates of the whole rodent nasal cavity have demonstrated esterase activity for acrylate esters [14]; we have investigated the relationship of the regional distribution of this activity for a representative acrylate ester, ethyl acrylate (EA) to its site-specific cytotoxicity following inhalation exposure.

The data from this study suggests that the AA released upon the hydrolysis of EA in the nasal cavity may contribute to the olfactory epithelial cytotoxicity observed following high dose inhalation exposure to EA vapor, but that AA does not solely mediate these effects. Furthermore, it is anticipated that the data derived from this study will be useful for modeling studies of the rodent nasal cavity and will facilitate the interspecies extrapolation of rodent toxicity results for human risk assessment.

MATERIALS AND METHODS

Male Fischer F344 rats (250–350 g at time of tissue collection) were obtained from Charles River Breeding Laboratories (Wilmington, MA) and housed two to three/cage with ad libitum access to feed and water. Nasal cavity tissues were collected from animals that had been killed by exsanguination under pentobarbital anethesia. The tissues, free of cartilage, were immediately weighed, frozen on dry ice, and stored at less than −60°C until homogenization and enzymatic analysis. The following regions of the nasal cavity were collected: (i) respiratory epithelium from the anterior ventral region of the septum combined with respiratory epithelium from the lateral walls (primarily from the nasoturbinate and the maxilloturbinate on each side); (ii) olfactory epithelium from the dorsal posterior region of the septum (grossly distinguishable by its yellowish color from the pink respiratory epithelium on the septum); and (iii) olfactory epithelium from the dorsal meatus region (the dorsal medial region of the

nasal cavity posterior to the incisive papilla). Representative tissue samples were fixed in formalin and examined microscopically to ensure that the correct tissues were being collected.

Tissue samples of each region were pooled from three to four animals and were homogenized in 25 vols. of pH 7.4 sodium phosphate buffer (0.1 M) with an ultrasonic cell disrupter. Aliquots of the whole tissue homogenate were incubated with 0.1–25 mM EA, and evaluated for carboxylesterase activity by HPLC analysis of released AA as previously described [15]. Michaelis-Menten parameters were determined by Eadie-Hofstee analysis using ENZPACK software (BIOSOFT, Cambridge, UK). The enzymatic nature of the ester hydrolysis was confirmed by including a boiled enzyme control of each tissue in each assay.

RESULTS AND DISCUSSION

Substrate inhibition of esterase activity was observed at EA concentrations exceeding 2.5 mM (Fig. 1) with homogenates from both regions of olfactory epithelium. This effect was most pronounced with the olfactory epithelium from the sensitive dorsal meatus region. The cytotoxicity-resistant respiratory epithelium exhibited modest esterase inhibition at concentrations exceeding 5 mM EA. Prior studies with homogenates of the mouse nasal cavity by Stott and McKenna suggest that the mechanism for this inhibition is probably based on either a solvent effect or binding to sulfhydryl residues on the enzyme [14], and that it extends to methyl acrylate and butyl acrylate. However, very similar results described for the carboxylesterease-mediated hydrolysis of ethyl butyrate (which does not bind to protein) suggest that a

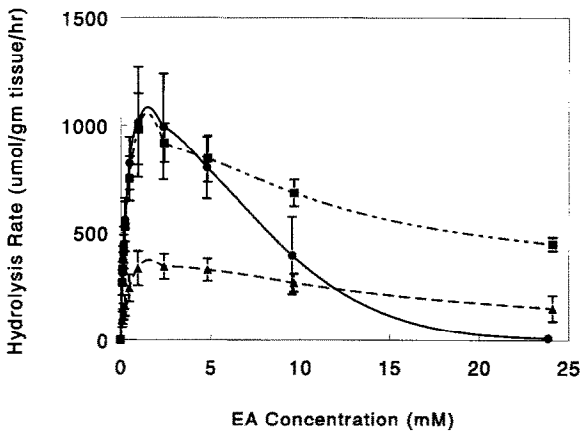

Fig. 1. The concentration-dependent rates of hydrolysis of EA catalyzed by esterases from various regions of the rat nasal cavity. Concentrations of EA in excess of 1 mM induced substrate inhibition of hydrolysis that was particularly marked in the olfactory epithelium isolated from the dorsal meatus region (●), relative to olfactory epithelium isolated from the septum (■) or respiratory epithelium (▲).

relatively stable enzymatic intermediate may combine with the high concentrations of substrate to prevent release of the acid product from the enzyme and therefore inhibit the reaction rate [16]. The significance of this high dose effect is unclear, since it is not known whether the amount of EA accumulating in specific regions of the nasal cavity would approach inhibiting concentrations. Modeling studies of the deposition and metabolism of EA in the rodent nasal cavity to address this issue are being pursued.

Due to the substrate inhibition at high EA concentrations, apparent Michaelis-Menten constants (Table I) were estimated based on substrate concentrations not exceeding 1 mM. Due to the multiplicity of carboxylesterases observed in other tissues, these enzymatic constants are described as 'apparent' values that may represent a composite functional capacity for the tissue based on the activity of several isozymes. The apparent K_m values for esterase activity were very similar for all three nasal cavity regions, 0.3–0.4 mM. However, the apparent V_{max} values were 3-times higher in the olfactory epithelium than in the respiratory epithelium on a tissue volume basis. No difference in esterase activity was observed between olfactory tissue collected from the sensitive dorsal meatus region versus the insensitive septal region. At relatively low substrate concentrations, the enzymatic kinetics assume pseudo first-order rate characteristics and a first-order rate constant can be estimated by V_{max}/K_m. This rate constant can be used to estimate a hydrolysis half-life in the tissue. These values, 0.06–0.07 s for olfactory epithelium and 0.2 s for respiratory epithelium, indicate that the rate of ester hydrolysis of EA in the rat nasal epithelium is very rapid, and a significant amount of the EA deposited in each region of the nasal cavity would be predicted to be hydrolyzed to release AA.

The cytotoxicity reported in the nasal cavity following inhalation exposure of rats and mice to EA is localized in the olfactory epithelium of the dorsal meatus [1]. This is the same area that was reported to exhibit cytotoxicity following inhalation exposure of rats and mice to acrylic acid [4] and of rats to methyl acrylate and butyl acrylate [2]. Since this region has a very high esterase activity, one explanation for the

TABLE I

APPARENT KINETIC CONSTANTS FOR THE ENZYMATIC ESTER HYDROLYSIS OF ETHYL ACRYLATE BY HOMOGENATES OF RAT UPPER RESPIRATORY TRACT TISSUE

Tissue source	K_m [a]	V_{max} [b] (tissue volume)	V_{max} [c] (protein)
Olfactory (dorsal meatus)	0.33 ± 0.06	1367 ± 356	14.5 ± 3.2
Olfactory (septum)	0.38 ± 0.02	1326 ± 180	13.2 ± 1.2
Respiratory	0.40 ± 0.11	443 ± 74	2.9 ± 0.2

Values are mean ± SD of three determinations from three different pooled tissue homogenates.

[a] μmol/ml tissue.

[b] μmol/ml tissue/h.

[c] μmol/mg protein/h.

cytotoxicity might be that the AA released upon hydrolysis mediates the cytotoxic effect. A review of the available data suggests that the released acrylic acid is responsible for some, but not all, of the toxicity observed following inhalation exposure to the esters. Stott and McKenna determined that the amount of EA deposited in the upper respiratory tract (URT, primarily the nasal cavity by their methodology) was approx. 66% of the inhaled concentration at a unidirectional flow rate of 53 ml/min and 40% at a flow rate of 105 ml/min [17,18]. The deposition was saturable with a sharp break in the deposition rate versus inhaled concentration curve at approx. 250 ppm EA. Pretreatment of the animals with an inhibitor of carboxylesterase significantly decreased the deposition rate of EA, indicating that '... approx. 50% of the loss of EA passing through the URT can be accounted for by the enzymatic hydrolysis of the absorbed compound within the URT' [18].

A comparison of the dosimetry and nasal epithelial toxicity observed at inhalation exposures of rats and mice provides some information on the mechanism of cytotoxicity (Table II). As an example, an inhalation exposure concentration of 75 ppm EA is a moderately cytotoxic dose level for the rat olfactory epithelium whereas exposures of 5 ppm EA and either 5 or 25 ppm AA did not induce nasal epithelial toxicity [1,4]. Extrapolation of the Stott and McKenna URT deposition data [17,18] as a function of flow rate to the resting normal rat minute volume (generally 150–200 ml/min) would predict that approx. 20 30% of the inhaled EA would be deposited in the nasal cavity. Approximately 50% of the EA deposited in the nasal epithelium would be hydrolyzed to AA [18] which would be the equivalent of a 6–9 ppm inhalation exposure to AA (assuming greater than 80% deposition of AA based on a personal communication from Dr. John Morris, University of Connecticut). However, inhalation exposure to an essentially equivalent dose level, 5 ppm AA, did not induce a toxic effect in the rat nasal cavity. These results indicate that the amount of AA released from the deposition and hydrolysis of EA in the nasal cavity is insufficient to explain the entire toxic response. By implication, the ester function must contribute at least part of the toxicity observed from the acrylate esters, although the site-specific concordance of the lesions observed following inhalation exposure to AA and its esters suggests that the parent acid makes some contribution to the cytotoxic effect. These conclusions are consistent with the potentiating effect of a carboxylesterase inhibitor on several functional indications of respiratory irritation induced by high dose (100–500 ppm) inhalation exposure of rats to EA, i.e., enhancement of an effect on respiratory frequency, tidal volume, and minute volume [19]. Although AA at the same dose levels also changed these parameters, the carboxylesterase inhibitor did not potentiate (or inhibit) the AA effect. These observations are consistent with different mechanisms for ester versus acid toxicity, even though a portion of the deposited ester is serving as a 'prodrug' for the acid. Prior studies from our laboratory have suggested that severe glutathione depletion correlates with the toxic effects of EA in other epithelial tissues [15], and this correlation may extend to the nasal cavity.

Mice are slightly more sensitive than rats to acrylate inhalation exposure (Table II), but the observation of very similar lesions with the same regional specificity and very

similar metabolism [14] would suggest that a similar dosimetry comparison for mice would result in the same conclusion. Previous comparative inhalation studies with acetone have indicated that chemical deposition in the nasal cavity of mice and rats is essentially equivalent [20].

The cytotoxicity observed in the dorsal meatus olfactory epithelium of mice and rats following subchronic and chronic inhalation exposure of these monomers is

TABLE II

SUMMARY OF INHALED CONCENTRATION VERSUS NASAL TOXICITY FOLLOWING INHALATION EXPOSURE TO ETHYL ACRYLATE AND ITS HYDROLYSIS PRODUCT, ACRYLIC ACID

Species	Compound	Inhaled concentration (ppm)	Estimated equivalent acrylic acid concentration (ppm)[a]	Exposure duration (months)	Principle toxic effects on nasal olfactory epithelium[c]
Rat	EA	5	0.4–0.6	24	none
Rat	EA	25	2–3	27	slight basal cell and glandular hyperplasia and respiratory metaplasia
Rat	EA	75	6–9	27	moderate basal cell and glandular hyperplasia and respiratory metaplasia
Mouse	EA	5	0.4–0.6	24	none
Mouse	EA	25	2–3	27	slight to moderate glandular hyperplasia and respiratory metaplasia
Mouse	EA	75	6–9	27	moderate to severe glandular hyperplasia and respiratory metaplasia
Rat	AA	5	NA[b]	3	none
Rat	AA	25	NA	3	none
Rat	AA	75	NA	3	slight focal degeneration
Mouse	AA	5	NA	3	very slight focal degeneration
Mouse	AA	25	NA	3	slight focal degeneration
Mouse	AA	75	NA	3	focal degeneration, respiratory metaplasia, and glandular hyperplasia

[a] Estimate of equivalent acrylic acid inhalation exposure is based upon the deposition efficiency of EA in the nasal cavity and the rate of hydrolysis of EA to AA by the nasal epithelium as described in the text. The range listed reflects the variation in normal rodent respiration.

[b] NA, not applicable.

[c] Summary description of toxic effects based on terminology used in the original publications [1,4].

followed by a replacement of the olfactory epithelium by chemically resistant 'respiratory' epithelium [1,2]. This adaptive response appears to be limited to dose levels that damage the basal cells of the olfactory epithelium. The origin of this ciliated 'respiratory' epithelium is not known, although it may be derived from the underlying glandular elements that are sensitive to a proliferative stimulus following acrylate exposure. Although the cells resemble respiratory epithelium microscopically, it is not clear whether they are exactly the same as normal respiratory epithelium. Progression of this lesion to neoplasia has not been observed following lifetime exposures. The basis for the sensitivity of the olfactory epithelium to organic acids and esters remains obscure. Air flow modeling studies of the nasal cavity [21] may provide insight for the preferential cytotoxicity observed in the olfactory epithelium of the dorsal meatus region relative to other regions of olfactory epithelium. Other explanations must be explored to explain the resistance of the respiratory epithelium to both acids and esters, since the 3-fold difference in ester hydrolysis rates between respiratory and olfactory epithelium observed in this study may not to be sufficient to explain this difference in cytotoxicity.

Given the marked difference in nasal cavity architecture between rodents and humans [22] and the regional sensitivity of the rodent olfactory epithelium for acrylic acid and ester exposure, a reasonably accurate estimate of the potential toxicity of various acrylate inhalation exposure scenarios will require additional dosimetry modeling. Studies are in progress to describe interspecies differences in metabolism, nasal cavity air flow, and regional chemical deposition of these monomers.

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REFERENCES

1 Miller, R.R., Young, J.T., Kociba, R.J., Keyes, D.G., Bodner, K.M., Calhoun, L.L. and Ayres, J.A. (1985) Chronic toxicity and oncogenicity bioassay of inhaled ethyl acrylate in Fischer 344 rats and B6C3F1 mice. Drug Chem. Toxicol. 8, 1–42.

2 Reininghaus, W., Koestner, A. and Klimisch, H.-K. (1991) Chronic toxicity and oncogenicity bioassay of inhaled methyl acrylate and *n*-butyl acrylate in Sprague-Dawley rats. Food Chem. Toxicol. 29, 329–339.

3 Morgan, K.T. and Monticello, T.M. (1990) Airflow, gas deposition, and lesion distribution in the nasal passages. Environ. Health Perspect. 85, 209–218.

4 Miller, R.R., Ayres, J.A., Jersey, G.C. and McKenna, M.J. (1981) Inhalation toxicity of acrylic acid. Fundam. Appl. Toxicol. 1, 271–277.

5 Keenan, C.M., Kelly, D.P. and Bogdanffy, M.S. (1990) Degeneration and recovery of rat olfactory epithelium following inhalation of dibasic esters. Fundam. Appl. Toxicol. 15, 381–393.

6 Lee, K.-P., Valentine, R. and Bogdanffy, M.S. (1992) Nasal lesion development and reversibility in rats exposed to aerosols of dibasic esters. Toxicol. Pathol. 20, 376–393.

7 Bogdanffy, M.S., Kee, C.R., Hinchman, C.A. and Trela, B.A. (1991) Metabolism of dibasic esters by rat nasal mucosal carboxylesterase. Drug Metab. Dispos. 19, 124–129.
8 Trela, B.A., Frame, S.R. and Bogdanffy, M.S. (1992) A microscopic and ultrastructural evaluation of dibasic esters (DBE) toxicity in rat nasal explants. Exp. Molec. Pathol. 56, 208–218.
9 Trela, B.A. and Bogdanffy, M.S. (1991) Cytotoxicity of dibasic esters (DBE) metabolites in rat nasal explants. Toxicol. Appl. Pharmacol. 110, 259–267.
10 Trela, B.A. and Bogdanffy, M.S. (1991) Carboxylesterase-dependent cytotoxicity of dibasic esters (DBE) in rat nasal explants. Toxicol. Appl. Pharmacol. 107, 285–301.
11 Dahl, A.R., Miller, S.C. and Petridou-Fischer, J. (1987) Carboxylesterases in the respiratory tracts of rabbits, rats and Syrian hamsters. Toxicol. Lett. 36, 129–136.
12 Bogdanffy, M. S, Randall, H.W. and Morgan, K.T. (1987) Biochemical quantitation and histochemical localization of carboxylesterase in the nasal passages of the Fischer-344 rat and the B6C3F1 mouse. Toxicol. Appl. Pharmacol. 88, 183–194.
13 Morris, J.B. (1990) First-pass metabolism of inspired ethyl acetate in the upper respiratory tracts of the F344 rat and Syrian hamster. Toxicol. Appl. Pharmacol. 102, 331–345.
14 Stott, W.T. and McKenna, M.J. (1985) Hydrolysis of several glycol ether acetates and acrylate esters by nasal mucosal carboxylesterase in vitro. Fundam. Appl. Toxicol. 5, 399–404.
15 Frederick, C.B., Potter, D.W., Chang-Mateu, M.I. and Andersen, M.E. (1992) A physiologically based pharmacokinetic and pharmacodynamic model to describe the oral dosing of rats with ethyl acrylate and its implications for risk assessment. Toxicol. Appl. Pharmacol. 114, 246–260.
16 Dixon, M. and Webb, E.C. (1979) Enzymes. Third Edition. Academic Press, New York, p. 129.
17 Stott, W.T. and McKenna, M.J. (1984) The comparative absorption and excretion of chemical vapors by the upper, lower, and intact respiratory tract of rats. Fundam. Appl. Toxicol. 4, 594–602.
18 Stott, W.T., Ramsey, J.C. and McKenna, M.J. (1986) Absorption of chemical vapors by the upper respiratory tract of rats. In: C.S. Barrow (Ed.), Toxicology of the Nasal Passages, Hemisphere, Washington, pp. 191–210.
19 Silver, E.H., Leith, D.E. and Murphy, S.D. (1981) Potentiation by triorthotolyl phosphate of acrylate ester-induced alterations in respiration. Toxicology 22, 193–203.
20 Morris, J.B. (1991) Deposition of acetone vapor in the upper respiratory tract of the B6C3F1 mouse. Toxicol. Lett. 56, 187–196.
21 Morgan, K.T., Kimbell, J.S., Monticello, T.M., Patra, A.L. and Fleishman, A. (1991) Studies of inspiratory airflow patterns in the nasal passages of the F344 rat and rhesus monkey using nasal molds: relevance to formaldehyde toxicity. Toxicol. Appl. Pharmacol. 110, 223–240.
22 Gross, E.A. and Morgan, K.T. (1992) Architecture of nasal passages and larynx. In: R.A. Parent (Ed.), Comprehensive Treatise on Pulmonary Toxicology. Volume I. Comparative Biology of the Normal Lung, CRC Press. Boca Raton, pp. 7–25.