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Pulmonary changes in rats following administration of 3-methylindole in cremophore EL

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Summary. 3-methylindole (3-MI) dissolved in the lipophilic carrier Cremophore EL was administered intraperitoneally to male, twelve-week-old Sprague-Dawley rats. Gross and histopathologic changes in the lungs were studied using light microscopy at three time-periods following administration: 16, 24, and 46 hours. Both 3-MI and Cremophore caused changes in bronchiolar epithelium at 16 hours. By 46 hours, Cremophore-injected rats showed no effects of the carrier; whereas, 3-MI rats showed severe lung changes characterized by airway epithelial and pulmonary vascular endothelial necrosis and sloughing, cellular infiltration by lymphocytes and macrophages, perivascular edema, alveolar edema, and lymph stasis. Grossly, the controls showed no effect of the carrier and none died during the studies. In contrast, 3-MI injected rats quickly became lethargic and displayed tachypnea, anorexia, and progressive respiratory distress. Two of five 3-MI rats in the final group died just prior to 46 hours. All of this group had grossly congested lungs and marked pleural effusion. The lesions and time course showed similarities to those observed in ruminants and mice. We conclude that 3-MI in Cremophore causes an acute progressive pneumonitis in rats and suggest that the rats may be a suitable model for 3-MI-induced and similar toxic lung diseases in domestic animals and people.

Key words: 3 - methylindole, Cremophore, Rat, Bronchiolitis

Introduction

The amino acid L-tryptophan can be metabolized to 3-methylindole (3-MI, skatole) in the intestinal tract of monogastric mammals and in the forestomach of ruminants (Yokoyama et al., 1977; Yokoyoma et al., 1979). In cattle, absorption of 3-MI from the rumen and its subsequent metabolism by lung mixed function oxidase enzymes is believed to be the cause of acute bovine pulmonary edema and emphysema (ABPE), a naturally-occurring respiratory disease (Carlson et al., 1975; Bray et al., 1979). Experimentally, administration of 3-MI causes acute respiratory disease in cattle (Carlson et al., 1972; Lekeux et al., 1985), sheep (Bradley et al., 1978; Ulvund, 1984), goats (Bradley et al., 1980; Huang et al., 1977; Dickenson et al., 1976; Mesina et al., 1984), and obstructive bronchiolitis in horses (Breeze et al., 1984; Turk et al., 1983). Until recently, it had been reported that laboratory animals were refractory to the pneumotoxic effects of 3-MI (Breeze et al., 1982; Carlson, Nocerini, and Breeze, 1984). However, Kiorpes et al., (1984) reported that 3-MI caused acute respiratory disease in rats. Subsequently, Turk et al. (1984, 1986) and Durham and Castleman (1985) reported dose responses and pathologic changes associated with 3-MI pneumotoxicity in mice.

In this paper we describe the histologic changes in the rat lung associated with intraperitoneal injection of 3-MI in Cremophore EL.

Materials and methods

Twenty-nine healthy, male Sprague-Dawley (SASCO/King, Inc., Omaha, NE) rats, approximately 270 g (12 weeks old), were chosen for study at three time periods: 16, 24, and 46 hours after parenteral administration of 3-MI. The rats were determined serologically negative to rat Coronavirus, Pneumonia Virus of mice, and mycoplasma by random sampling of rats at the source.

Fifteen rats comprised the 16 hour toxicity group (group 1: 8 test, 7 control rats). Prior to study, these rats were housed in polycarbonate cages (30x20x14 cm) fitted

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with stainless steel lids and bedded with hardwood shavings. Because they were included in a nutritional study (data not reported here), they were fed a torula-yeast based diet supplemented with vitamin E and selenium and containing cod-liver oil as a fat and vitamin A source (Hafeman and Hoekstra, 1977). Forty-eight hours prior to study, an identical diet but without cod-liver oil was substituted. Then rats were placed in individual, glass desiccation jars, each with its own air supply for respiratory gas analysis, as described elsewhere (Hafeman and Hoekstra, 1977). Food and water were withheld throughout the 16 hours of the experiment. Only the histopathologic findings in this experiment are reported here. All Group I studies began at approximately 6 am. Group II rats (n = 14) were part of a biochemical experiment the results of which have been reported previously (Kiorpes et al. 1984). Six rats served as controls and eight rats comprised the 3-MI experimental group (test). Two test and three control rats were sacrificed at 24 hours (one test rat died prior to 24 hours and was eliminated from subsequent analysis); the remaining eight either died (n = 2) by or were sacrificed (n = 6) at 46 hours. These rats were housed in hanging metal wire cages of similar size as before with no bedding. A standard laboratory chow (Purina Rat Chow, St. Louis, MO) was fed ad lib. Food was withheld from this group for 12 hours prior to injection. However, after injection, these rats were returned to their cages and food and water were available at all times. These studies began at approximately 9 am. Surviving rats in Groups I and II were euthanized with an overdose of pentobarbital sodium (Rugby Inc., Rockville Center, NY) and necropsied immediately; rats that died spontaneously were necropsied within 15 minutes of death.

3-MI (99%, crystalline) was purchased commercially (Sigma Chemical Co., St. Louis, MO) and prepared in the laboratory as a 2% (w/v) solution in a solvent of 10% (v/v) Cremophore EL (Sigma) in double distilled, deionized water. Cremophore is a lipophilic carrier commonly used in human medicine with no reported pneumotoxic effects. Preparation of the 3-MI plus Cremophore solution required gentle heating and constant stirring. Overheating caused the 3-MI to become an opaque, white suspension in the Cremophore and was not used. Test and control rats were weighed to the nearest gram and administered either freshly-prepared 3-MI solution at a rate of 400 mg/kg or an equivalent volume of 10% Cremophore EL alone as an intraperitoneal (IP) bolus injection. Based on 20 mg/ml concentration, the volumes injected were approximately 5 ml. Three rats served as internal controls and were not injected.

The 400 mg/kg dosage was based on prior dose-response studies at 200, 250, 300, 350, 400, and 450 mg/kg body weight using male Wistar and Long Evans rats (average weight 725 g). Results showed that 300 to 400 mg/kg 3-MI in Cremophore produced dyspnea and death in 48 to 72 hours. Subsequently, a time-to-death study using six rats of the same size and strain as reported here was performed using the 400 mg/kg dose. Death occurred in four of the six rats with a mean time to death of 45 hours.

Lungs were fixed in situ with the rats placed in dorsal recumbency. A midline incision was made from the umbilicus through the sternum. The color and consistency of the lungs and the presence of pleural fluid were noted. The right and left ventricles of the heart were opened at the apex and ice-cold heparinized saline was slowly perfused into the pulmonary artery at a constant rate using a syringe. The trachea was cannulated, and Bouin's fixative was instilled into the lung at a constant pressure of 20 cm H_20. When no more fixative entered, the lungs were removed from the chest, and 3 mm thick, longitudinal, near-saggital sections were cut and placed in fresh Bouin's solution for 24 hours. The lung sections were then washed twice in 70% ethanol and embedded in paraffin blocks. Five-micron thick sections were prepared and stained with hematoxylin and eosin (H and E) for examination with a light microscope.

The following histological parameters were used to assess 3-MI toxicity: airway epithelial and vascular endothelial changes, lymphocytic infiltration, perivascular edema, alveolar edema, evidence of lymph stasis, alveolar wall cellularity, and numbers of alveolar macrophages and interstitial polymophonuclear leucocytes (PMNs). Each parameter except the last three were assigned a grade from 0 (no changes) to +++ (severe changes). Specifically, for airway epithelium, 0 = no pathologic changes; + = occasional, (mild) epithelial exfoliation; ++ = (moderate) exfoliation of approximately half the epithelium with nuclear pyknosis and cytoplasmic vacuolization; +++ = (severe) extensive exfoliation of the epithelium with necrosis, vacuolization, and pyknosis. For endothelium, 0 = no pathologic changes; + = (mild) occasional, focal endothelial exfoliation; ++ = (moderate) exfoliation of endothelium affecting approximately half the vessels; +++ = (severe) exfoliation of the endothelium in the majority of vessels. All categories of airways and vasculature were included in the evaluation of each slide. The other parameters were evaluated similarly and graded normal through severe. Evidence of lymph stasis was based on the presence and extent of swollen lymphatic vessels in the adventitia and interstitium. Because histologic changes were more subtle, the grading system at 16 hours was modified with respect to airway epithelium: 0 = no pathologic changes; + = swelling and vacuolization of occasional Clara cells with intact ciliary epithelium; ++ = swelling, vacuolization, and occasional necrosis of approximately half of the Clara cells with intact ciliary epithelium; +++ = necrosis of most Clara cells with focal detachment of ciliary epithelium from the basement membrane.

Indices reflecting the numbers of alveolar macrophages, numbers of alveolar wall PMNs, and alveolar wall cellularity (except for PMNs) were established. For the latter two, ten representative hexagonal alveoli were chosen using a method for random selection of fields. Each section was projected onto a screen using a projection microscope (XM-150, Kramer Scientific Corp.) and a 63 x objective (Karl

Zeiss, West Germany). Distance between the objective and the screen was 230 cm producing a 1500X linear magnification. The number of cells within the septa of each chosen alveolus were counted as identified by their nuclei with H and E. Blood cells were excluded, and tissue PMNs were counted separately. Alveolar macrophages were quantitated by counting all such cells with a visible nucleus that were present in each of ten random high-power fields (hpf) of uniform alveolar tissue, using a light microscope with a 40X objective and 400X total magnification. The quantitative data were expressed as means and standard deviations.

For statistical evaluation grades of pathologic changes, (0 to +++) were transformed into numerical scores (see Tables 1 and 2) and analyzed using a non-parametric two-sample rank procedure (Mann-Whitney). Because of the small sample size, the 24 hour test group was not included in the statistical comparisons. Pooled 24 and 48 hour control group data were used when there was no difference between the two groups. Significant differences in the parametric variables (alveolar wall cellularity, and alveolar wall PMNs) at $p \leqslant 0.05$ were determined using Student's two sample t-test (Ryan T.A., Joiner B.L. and Ryan B.F., Minitab Student Handbook, Doxbury Press, First Edition, 1976).

Results

Cremophore EL alone had no apparent clinical or behavioral effect on rats. In contrast, 3-MI in Cremophore produced a state of torpor in all rats within minutes of injection, which continued throughout the observation periods with no remission. Test rats with access to food and water neither ate no drank. Increased tear production (epiphora) was a constant finding; in most rats the tears appeared blood-tinged. A sero-hemorrhagic crusting was common around the nares in 3-MI treated rats. An increased respiratory rate was noted in test rats as early as four hours post-injection and continued until just prior to death. Death was preceded by labored, open-mouth breathing. No control animals died.

Gross post-mortem inspection of control rats showed no significant changes except focal, serosal intestinal hemorrhages associated with injection. The abdominal viscera of the test rats were similarly unremarkable, except that mild liver congestion was observed in two rats at 46 hours; most likely reflecting passive congestion. The predominant pathologic changes in test rats were confined to the chest: the lungs were mildly to moderately congested at 16 and 24 hours and severely congested at 46 hours. Pleural effusion was present in all test rats by 24 hours.

The histologic findings are summarized in Tables 1 and 2. At 16 hours, epithelial exfoliation, alveolar edema, and lymph stasis were not observed in either test or control rats. Changes in vascular endothelium were occasionally observed with mild exfoliation in control and mild to moderate exfoliation in test rats. These changes were limited to pulmonary veins and venules. Changes in airway epithelium were the most consistent findings in both test and control rats at 16 hours when compared with non-injected internal controls not reported here (Figs. 1a, b). These changes were characterized by mild vacuolization and swelling, increased mitotic activity, and occasional necrosis of Clara cells with focal flattening of the epithelium (Figs. 2a, b). There were minimal changes in ciliated airway cells. These findings in control rats were diminished by 24 hours and were insignificant by 46 hours, but persisted among test rats and became more severe as described below.

At 16 hours there was mild lymphocytic infiltration throughout the lung tissue in 4 out of 7 control, and 7 out of 8 test rats; similarly, mild to moderate perivascular edema was observed in both test and control rats. Numbers of alveolar macrophages were significantly increased at 16 hours in 3-MI treated rats compared to controls, and rose dramatically by 46 hours. The PMN index did not differ between test and control rats at 16 hours, but both 16 hour test and control indices were significantly increased over 24 and 46 hour controls.

Mild airway epithelial and vascular endothelial exfoliation at 24 hours progressed to severe by 46 hours in 3 out of 5 test rats (Figs. 3b, 4); whereas, the lungs of control rats by 46 hours were normal (Fig. 3a). Within each rat, the degree of severity was uniform; however, severity of epithelial and endothelial changes did not appear to be related to each other. Some rats had mild vascular and severe airway changes and vice-versa. Clara cells in all test rats were necrotic; most were unidentifiable or absent. The degree of intra-pulmonary lymphocytic infiltration in test rats at 46 hours was similar to that seen in both 3-MI and control rats at 16 hours (Fig. 5). However, at 24 and 46 hours no lymphocytic infiltration was noted among controls. Perivascular edema was uniformly severe, and alveolar edema (Fig. 4) was moderate to severe in all 46-hour test rats. At this time, mild lymph stasis was evident. The lungs of test rats at 46 hours were significantly more cellular than controls with increased macrophage and alveolar wall cellularity indices. Alveolar wall cellularity indices appeared to increase progressively from 16 to 46 hours, suggesting an intensifying inflammatory response.

The tissue preparation used here did not allow careful evaluation of type I squamous cells (type I cells) and type II granular pneumocytes (type II cells) of the alveolar epithelial lining; thus, no quantification was done. However, some Type II cells could be identified by their characteristic shape, nucleus to cytoplasm ratio, and location. Occasional mitotic figures and groups of 3-5 cells suggested type II cell proliferation and hyperplasia at 46 hours in 3-MI injected rats.

Table 1. Histopathologic Changes in Rat Lungs Induced by 3-Methylindole in Cremophore EL in Comparison with Cremophore EL alone at 16 hours post-injection [1-3].

Histological Parameter	Treatment:	3-Methylindole in Cremophore EL (Test, n = 8)									Cremophore EL Only (Control, n = 7)							
	Rat	1	2	3	4	5	6	7	8	Median/ Mean	Rat	9	10	11	12	13	14	15	Median/ Mean
Airway Epithelial Change		++	+	+	+	+	+	++	+	1.0		+	+	+	+	0	0	+	1.0
Endothelial Exfoliation		+	++	0	0	++	0	+	0	0.5		0	0	0	+	0	0	+	0
Lymphocytic Infiltration		+	+	+	0	+	+	+	+	1.0		0	+	0	++	0	+	+	1.0
Perivascular Edema		+	+	+	++	+	+	+	+	1.0		+	+	++	+	0	+	+	1.0
Lymph Stasis		0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0
Alveolar Edema		0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0
Alveolar Macrophages		2.4	4.20	1.80	3.30	4.20	2.30	2.30	3.80	3.0 ± 1.0*		1.3	1.6	1.4	1.8	1.2	2.1	1.0	1.4 ± 0.4
Alveolar Wall Cellularity		8.7	10.4	10.6	6.7	8.8	8.8	11.0	8.9	9.2 ± 1.4		9.7	8.9	7.2	9.3	6.7	9.6	8.3	8.5 ± 1.2
Alveolar Wall PMN		0.8	0.7	0.7	0.8	0.1	0.6	0.6	1.5	0.7 ± 0.4		0.1	0.4	0.7	0	0.3	0.7	0.4	0.4 ± 0.3

1. 400 mg/kg 3-methylindole in Cremophore EL injected intraperitoneally.
2. Individual values, group medians for non-parametric values, group means and standard deviations for parametric variables; sample sizes given in parenthesis.
3. 0 = absent (0); + = mild (1); ++ = moderate (2); +++ = severe (3);

*Significantly different from controls at $p \leqslant 0.05$.

Table 2. Histopathologic Changes in Rat Lungs Induced by 3-Methylindole in Cremophore EL in comparison with Controls given Cremophore EL only at 24 and 46 hours post-injection[1-4].

Histological Parameter	Treatment:	3-Methylindole in Cremophore EL (Tests)										Cremophore EL Only (Controls)								Mean Pooled Controls
		24 hours (2)				46 hours (5)						24 hours (3)				46 hours (3)				
	Rat	16	17	18	Median/ Mean	Rat	19	20	21	22†	23†	Median/ Mean	Rat	24	25	26	Median/ Mean	Rat	27	28	29	Median/ Mean	(6)
Airway Epithelial Exfoliation		+	D	+	1.0		+	+	+	+	+	3.0**		0	+	0	0		0	0	0	0	0
Endothelial Exfoliation		+	D	+	1.0		+	++	+	+	+	3.0*		0	0	+	0		0	+	+	1	0.5
Lymphocytic Infiltration		0	D	0	0		+	+	+	0	+	1.0**		0	0	0	0		0	0	0	0	0
Perivascular Edema		+	D	++	1.5		+	+	+	+	+	3.0**		+	0	+	1.0		0	0	0	0	0
Lymph Stasis		0	D	−	0.5		+	+	0	−	+	1.0**		0	0	0	0		0	0	0	0	0
Alveolar Edema		0	D	0	0		+	+	+	+	+	3**		0	0	0	0		0	0	0	0	0
Alveolar Macrophages		2.1	D	4.2	3.2 ± 1.5		18.2	26.8	9.2	5.8	6.0	13.2 ± 9.1**		0.4	1.2	0.4	0.8 ± 0.5		1.4	3.2	3.8	2.8 ± 1.3	1.7 ± 4
Alveolar Wall Cellularity		10.1	D	9.7	9.9 ± 0.3		15.1	11.5	11.9	11.6	9.7	12.4 ± 2.0*		11.1	11.4	8.8	10.5 ± 1.4		6.8	10.2	9.4	9.5 ± 0.7	9.6 ± 0.6
Alveolar Wall PMN		14.2	D	5.2	9.7 ± 6.4		2.6	12.0	10.4	4.3	3.3	6.5 ± 4.45		5.8	2.8	2.4	3.7 ± 1.9		0.2	0	0.6	0.4 ± 0.3	2.0 ± 2.2

1. 400 mg/kg 3-methylindole in Cremophore EL injected intraperitoneally.
2. Individual values, group medians for non-parametric values or means and standard deviations for parametric values; sample sizes given in parenthesis.
3. 0 = absent (0); + = mild (1); ++ = moderate (2); +++ = severe (3);
4. Means of pooled controls calculated when no significant difference between 24 and 48 hours.

* Significantly different from pooled controls at $p \leqslant 0.05$.
** Significantly different from matched and pooled controls at $p \leqslant 0.05$.
*** Significantly different from matched controls at $p \leqslant 0.05$.
† Died at 45 hours post injection.
D = Died within 6 h of injection; not suitable for evaluation.

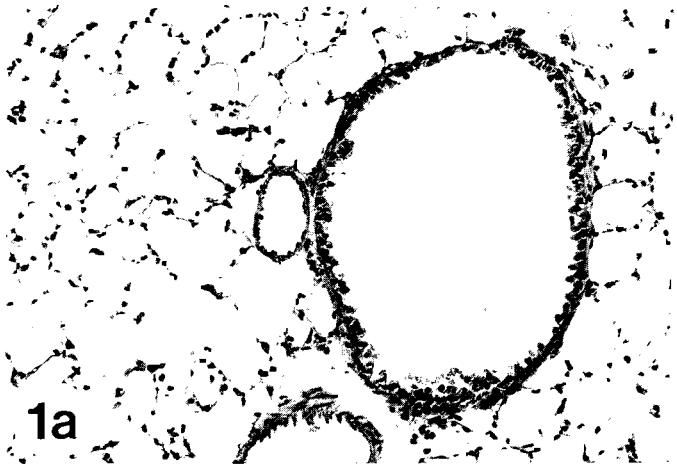
Figs. 1a,b. Normal bronchiole from a control rat 16 hours after administration of Cremophore EL. Note Clara cells protruding into the lumen and no evidence of edema (1a). **1b,** bronchiole of a test rat 16 hours post-administration of 3-MI in Cremophore. The epithelium is flattened, and Clara cells are inconspicuous (× 110).

Fig. 2a,b. Swelling and vacuolization of bronchiolar Clara cells in 16-hour control rat **(2a).** Note the halo effect around nuclei and a complete layer of basal cell nuclei (arrows) near the basal lamina. **2b,** Clara cell mitosis (arrows) in flattened bronchiolar epithelium of a 16 h test rat (× 435).

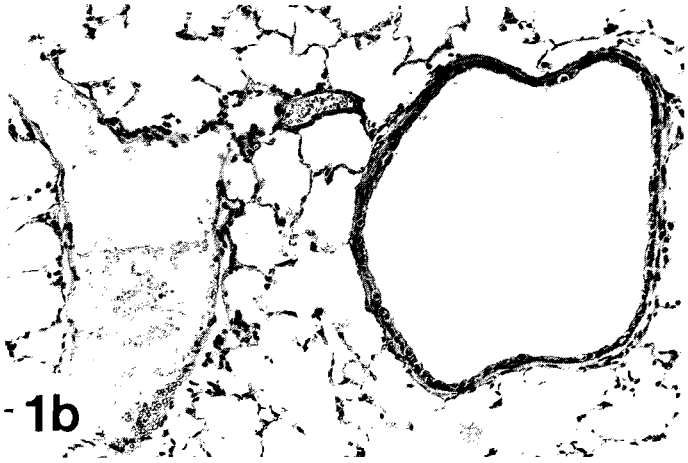
Fig. 3a,b. Bronchus from normal control rat 46 hours after administration of Cremophore **(3a)** and from a test rat 46 hours after administration of 3-MI in Cremophore EL **(3b).** The ciliated epithelium is intact in the control rat and the adventitia (Ad) shows no signs of edema or lymphocytic infiltration. Various stages of epithelial necrosis and detachment are noted in the test rat. Note partial epithelial detachment from the basal lamina, apparent lack of cilia, and adventitial edema **(3b)**.

Fig. 4. Lung tissue from a 46-hour test rat. Note the extensive bronchiolar epithelial exfoliation, a vein with endothelial detachment (arrows), perivascular adventitial (Ad) edema, and alveolar edema (× 110)

Fig. 5. Lymphocytic, perivascular cuffing in the lung of a 46-hour test rat (× 435)

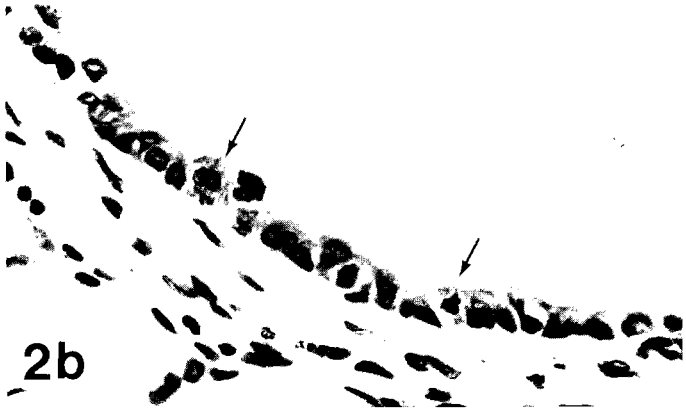


1a



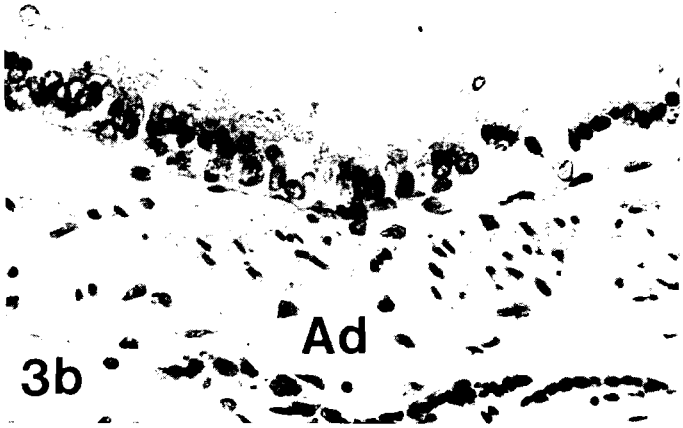
1b

2a

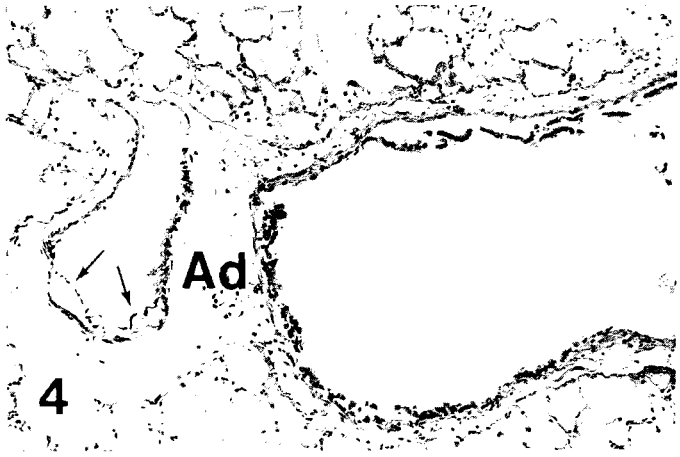


2b

3a



3b



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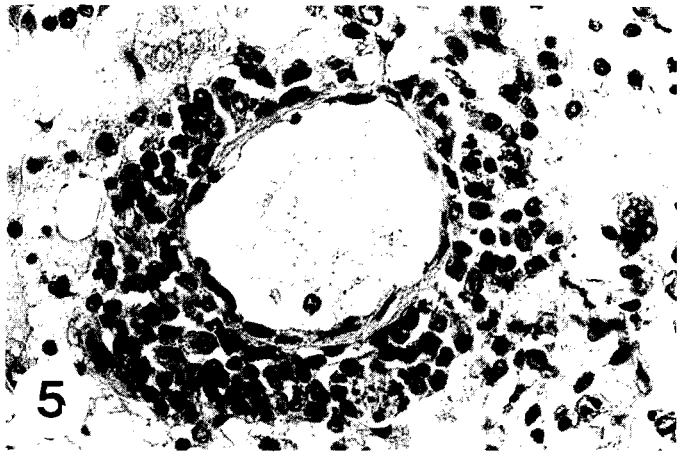

5

Discussion

The results of these experiments have shown that administration of 3-MI to rats causes depression, epiphora, nasal discharge, and progressive respiratory distress. Lethargy has been reported to occur in mice within five minutes of 3-MI administration (Turk et al., 1984), but not in any other species. The reasons for this response are not known. Epiphora has not been described previously following 3-MI administration. The bloody tint may be porphyrins in the tears (chromodacryorrhea), a well recognized sign of toxicity, or may be the result of capillary endothelial damage. The nasal crusting we observed may be evidence of acute mucosal inflammation. We did not include the upper airways in the histological evaluation; however, olfactory epithelial necrosis has been reported in mice administered 3-MI (Turk et al. 1986). Hydrothorax has been observed in mice (Durham and Castleman, 1985), but not in any other species.

In our rats, respiratory distress at 16 hours and death between 24 and 46 hours was a consistent finding. These mortality data differ from those reported for weanling, outbred, CD-1 mice, where 3-MI (500 mg/kg IP in corn oil) caused death in only 3 out of 42 mice by 48 hours (Durham and Castleman, 1985). In adult C57BL/6N mice administered 400 mg/kg 3-MI in corn oil, (n = 12) no deaths were observed by 24 hours but bronchiolar epithelial necrosis was present (Turk et al., 1984); however, at dosages of 600 and 800 mg/kg, 1 out of 5 and 5 out of 5 mice, respectively, died within 20 hours of injection (Turk et al., 1984). These data suggest that rats may be more sensitive than mice to the effects of 3-MI. This is surprising based on previous reports that rats were resistant to 3-MI (Breeze and Carlson, 1982) and data showing that rat lung microsomes produce relatively small amounts of activated 3-MI (Nocerini, et al., 1985). Additional studies are needed to assess the effect of different 3-MI carriers, animal strains, age, and diets before further generalizing between species.

The increased respiratory rates observed in rats following 3-MI administration have also been noted in other species. In weanling mice, increased respiratory rates were recorded within one hour of 3-MI administration and dyspnea was evident from 18 to 36 hours (Durham and Castleman, 1985). In goats administered 200 mg/kg 3-MI per os, increased respiration was reported by four to six hours (Mesina et al., 1984). In horses, increased respiration was reported at 24 hours following oral administration of 3-MI (Derksen et al., 1982). The stimulus for increased respiration has not been investigated in rodents; however, in horses, vagotomy abolished the response, suggesting that the tachypnea of 3-MI toxicity was mediated by pulmonary irritant receptors rather than by hypoxemia (Derksen et al., 1982).

The histologic findings in this study demonstrate both similarities and differences between rats and other species with respect to 3-MI's pneumotoxic effects. Alveolar pulmonary edema has been reported in ruminants (Huang et al., 1977; Mesina et al., 1984; Ulvund, 1984; Lekeux et al., 1985; Bradley et al., 1978; Carlson et al., 1975; Dickenson et al., 1976; Bray et al., 1979), and as a terminal event in horses (Turk et al., 1983) and weanling mice (Durham and Castleman, 1986). It appears to be associated with severe respiratory distress and death in rats. However, pulmonary edema was not observed in adult mice despite bronchiolar epithelial damage (Turk et al., 1986). The time course of edema formation differs between species. In ruminants, edema is an early clinical and histologic feature (Mesina et al., 1984; Huang et al., 1977); whereas, in rats, neonatal mice, and horses, it appears later. The mechanism of 3-MI induced pulmonary edema is not known for certain. Increased pulmonary vascular pressure has been suggested as a possible mechanism in cattle (Atwal and Persofsky, 1984a, 1984b), although this has been shown not to be the case in goats (Mesina et al., 1984). Decreased plasma oncotic pressure (hypoproteinemia) or increased hydrostatic pressure could increase pulmonary transepithelial fluid flux; however, this type of edema is usually a transudate containing small amounts of protein (Bernard and Brigham, 1986). Our histopathologic findings in rats suggest a proteinaceous edema, as might occur with damage to capillary endothelial and alveolar epithelial cells. It could be argued that we risked creating artifacts by not controlling perfusion pressure more precisely; however, we believe that the degree of alveolar flooding in 46 hour test rats cannot be explained by variations caused by careful hand-perfusion of the vasculature.

In rats, the primary cell types affected by 3-MI appear to be the vascular endothelium and airway epithelium, particularly Clara cells. The same is true of weanling mice and ruminants; however, type I alveolar epithelium is also primarily affected in these two species. Because of the limitations of our preparation, we did not evaluate the alveolar type I cells, and it remains to be shown whether 3-MI induces alveolar epithelial necrosis in rats. Apparently, type I cells are not affected by 3-MI in horses (Turk et al., 1983) and adult mice (Turk et al., 1986). Species differences in type I epithelial susceptibility to 3-MI may explain why alveolar edema has not been observed in adult mice and only in horses in extremis. Type II cell hyperplasia, as suggested in our 3-MI rats at 46 hours, has been previously described in mice (Durham and Castleman 1985). Such changes may indicate type I cell damage, as type II cells proliferate prior to replacement of injured type I cells.

Infiltration by macrophages and PMNs into the perivascular, peribronchiolar, and intraalveolar spaces is a response to inflammation and cellular necrosis, and has been reported in weanling mice, horses, and ruminants following 3-MI administration. The elevated PMN indices at 16 hours in both control and test rats compared with uninjected controls was surprising, and suggests that Cremophore alone may induce early, mild, reversible inflammatory changes in the lung parenchyma. Lymphocytic infiltration was related to pneumotoxicity in the 46 hour study. The seemingly random appearance of these latter infiltrates in 16 hour rats suggests the presence of viral or mycoplasmal respiratory disease in some subjects or an irritant effect of Cremophore alone evident only

acutely. Although these rats were from clean stock, specific tests were not performed at post-mortem to totally rule out the possibility of pulmonary infection.

The findings that 3-MI causes severe changes in rat Clara cells agree with findings in mice (Durham and Castleman, 1985; Turk et al., 1986), ruminants (Huang et al., 1977; Bradley et al., 1978), and horses (Breeze, Brown, and Turk, 1984), and they further support the concept that pneumotoxicity of 3-MI occurs secondary to its metabolic activation via cytochrome P-450-dependent pathways. Cytochrome P-450 b and P-450c have been recently reported in the Clara cells and type II pneumocytes of male Sprague-Dawley rats (Keith et al., 1987). These same authors have shown that 3-methylcholantrene can induce P-450c immunoreactivity in phulmonary endothelial cells of rabbits and have suggested that pulmonary edema may result from endothelial cell damage secondary to P-450c induction. The ability of 3-MI to induce these metabolizing enzymes and cause pulmonary edema in rat lung cells remains to be demonstrated.

That Cremophore EL produced mild, early changes in the vascular endothelium and airway epithelium of rats was an unexpected finding. We considered the possibility that these changes were artifacts of preparation; however, since the Cremophore-induced changes in control rats appeared to resolve by 46 hours, this interpretation seemed unlikely. It is possible that the different handling of the 16 hour rats contributed to these changes in the controls; however, we propose that Cremophore alone is suspect. Cremophore EL is a polyoxylated castor oil derivative and is commonly used in human medicine as a parenteral or topical carrier of lipophilic compounds. Side effects, such as anaphylaxis, are rare (Ptachcinski et al., 1985), although ricinoleic acid, a component of castor oil, can effect the gastrointestinal tract (Racusen and Binder, 1979). We observed neither GI effects nor anaphylaxis in the present or any of our pilot studies. Cremophore given by rapid IV infusion has caused pulmonary hypertension and intravascular hemolysis in goats and cattle (Breeze, Laegrid and Olcott, 1984; Atkinson et al., 1977). Recently, Cremophore administered to rats has been associated with renal vasoconstriction (Thiel, et al., 1986) and tubular vacuolization (Kone, et al., 1986). Although there are no reports of pulmonary vascular endothelial and airway epithelial changes following administration of Cremophore, our data suggest that such changes may occur. The specific pulmonary cells and P-450 effects of Cremophore in rats should be subject to further investigation.

From the findings in these experiments, we conclude that 3-MI in 10% Cremophore EL injected IP into Sprague-Dawley rats causes acute respiratory distress and lung damage similar in many respects to ABPE in ruminants. We suggest that the rat is a suitable laboratory animal model for study of 3-MI-induced pneumotoxicity.

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