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Pathophysiologic study of 3-methylindole-induced pulmonary toxicosis in immature cattle

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SUMMARY

In 5 Friesian calves given 3-methylindole (3-MI) (100 mg/kg once a week for 8 weeks, except calf 4, given a 50 mg/kg dose on weeks 3 to 8), pulmonary function (PF) values and arterial blood gas tensions (Pa_{O_2} and Pa_{CO_2}) were measured 24 hours after dosing was done and were correlated with clinical, biochemical, and pathologic changes.

Three of the calves (No. 1, 2, and 3) showed acute respiratory distress syndrome 24 hours after the first 3-MI treatment, with a large increase in respiratory frequency, minute viscous work, and Pa_{CO_2} and a large decrease in tidal volume, dynamic lung compliance, and Pa_{O_2}. They died 36, 38, and 84 hours after dosing.

Pulmonary function changes were compatible with the severe pulmonary edema and alveolar damage observed at necropsy. The 2 other calves, after they were given the 1st dose, showed only subacute respiratory distress syndrome with less severe changes in PF values recorded at 24 hours. Furthermore, they became progressively more tolerant to the 2nd, 3rd, and 4th weekly treatments, and showed base-line PF values after the 5th weekly treatment. Pathologic changes were not observed in lung biopsy material from these 2 animals at 2 and at 12 weeks after the 8th (or last) 3-MI treatment.

3-Methylindole (3-MI), a metabolite of the amino acid L-tryptophan, is known to cause alveolar epithelial cell and endothelial cell damage in cattle.[1-4] It has, therefore, been incriminated in the cause of certain respiratory tract diseases.

Clinical changes of this pulmonary toxicosis vary from moderate dyspnea to acute respiratory distress syndrome (RDS), depending on the initial dose of 3-MI, the route of drug administration, and the individual sensitivity.[1-4]

Logan et al[3] suggested that repeated oral administrations of 3-MI in cattle provided a good experimental model of diffuse pulmonary fibrosis. However, the effects of 3-MI on the pulmonary function have not yet been reported in immature cattle. Mechanics of breathing have been studied in calves with airways diseases,[5-7] but not in those with conditions involving mainly the alveoli.

The purpose in the present work was to determine changes in pulmonary function and gas exchange induced by 3-MI given repeatedly to immature cattle fed hay and concentrate and to correlate these functional changes with clinical, biochemical, and pathologic changes.

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Materials and Methods

Five female Dutch Friesian calves (5 months of age and weighing between 120 kg and 148 kg) were used. Seemingly, they were free of respiratory tract problems and were considered to be healthy, as determined by clinical examination. Each calf was fed 2 kg of concentrate each day, and hay and water were made available ad libitum.

Once a week for a maximum of 8 weeks, 3-MI was administered via a stomach tube at the dosage of 100 mg/kg of body weight, in the form of drug suspension in 5 L of water (Table 1). Calf 4, however, developed acute RDS after the 2nd 3-MI treatment, and thus, the dosage was reduced to 50 mg/kg for the 3rd through 8th weeks.

Clinical examination was done twice a day for each calf. Body weight and thoracic perimeter were determined once a week. Once a week, samples of venous blood were obtained at 6 hours after the calves were given 3-MI; these were analyzed for plasma 3-MI concentration.[8]

Pulmonary function values and arterial blood gas tensions were measured 24 hours before the 1st 3-MI treatment (base line) and 24 hours after each dosing. These measurements were done (calves were not sedated) in an air-conditioned room, using techniques, methods, and standard procedures.[9,10]

The following values were measured: respiratory frequency (f), inspiratory time/total time of the breathing cycle (t_I/t_{TOT}), mean inspiratory and expiratory airflow, tidal volume (V_t), minute volume ($\dot{V}e$), the lowest intrapleural pressure (Ppl) during inspiration, the highest Ppl during expiration (Pplmax), peak-to-peak Ppl changes (maxdPpl), Ppl at the functional residual capacity level (Ppl_{frc}), dynamic lung compliance (Cdyn), total pulmonary resistance (R_L), viscous work of breathing, minute viscous work ($\dot{W}vis$), pH, arterial oxygen tension (Pa_{O_2}), and carbon dioxide tension (Pa_{CO_2}). The alveolar-arterial oxygen difference ($A\text{-}aDO_2$) was calculated.[11] In the surviving calves, No. 4 and 5, multiple lung biopsies were done through the rib cage, at 2 and 12 weeks, respectively, after the calves were last treated with 3-MI and were submitted for pathologic examination.

TABLE 1—Changes in healthy Friesian calves given 3-MI

Calf No.	Dose of 3-MI in mg/kg (once per week) × No. of doses	Clinical condition		Plasma 3-MI concentration		
		After the 1st 3-MI treatment	After the last 3-MI treatment	Base line (μg/ml)	After the 1st 3-MI treatment (μg/ml)	After the last 3-MI treatment (μg/ml)
1	100 × 1	ARDS (died)	...	< 0.5	1.6	...
2	100 × 1	ARDS (died)	...	< 0.5	1.7	...
3	100 × 1	ARDS (died)	...	< 0.5	3.1	...
4	100 × 2 50 × 6	SRDS	Base line	< 0.5	2.3	< 0.5
5	100 × 8	SRDS	Base line	< 0.5	2.5	1.5

ARDS = Acute respiratory distress syndrome. SRDS = Subacute respiratory distress syndrome.

TABLE 2—Pulmonary function in five healthy Friesian calves at 24 hours after they were given the 1st dose of 3-MI

Values (unit of measurement)	Base line (calves 1 to 5)	After treatment	
		Calves 1, 2, and 3	Calves 4 and 5
f (min^{-1})	31(2)	103(24)	47(7)
t_I/t_{TOT}	0.47(0.02)	0.48(0.04)	0.49(0)
$\dot{mVi}$ (1/s)	1.3(0.1)	3.2(0.4)	1.6(0.1)
$\dot{mVe}$ (1/s)	1.2(0.1)	3.0(0.6)	1.5(0.1)
V_t (l)	1.20(0.20)	0.88(0.10)	0.95(0.10)
$\dot{V}e$ (l/min)	37(5)	89(12)	45(6)
Pplmin(−) (kPa)	1.29(0.16)	2.79(0.65)	1.22(0.17)
Pplmax(−) (kPa)	0.57(0.13)	0.30(0.28)	0.47(0.12)
maxdPpl (kPa)	0.72(0.08)	2.49(0.30)	0.75(0.09)
Ppl_{fre}(−) (kPa)	0.68(0.07)	0.88(0.32)	0.65(0.05)
Cdyn (l/kPa)	2.85(0.46)	0.61(0.23)	2.25(0.21)
R_L (kPa/l/s)	0.16(0.04)	0.16(0.03)	0.15(0.05)
Wvis (J)	0.43(0.12)	0.92(0.28)	0.45(0.05)
$\dot{W}$vis (J/min)	13.4(2.9)	91.0(23.0)	21.6(6.8)
HR (min^{-1})	78(4)	100(12)	85(8)
pH	7.42(0.01)	7.33(0.02)	7.41(0.02)
Pa_{O_2} (kPa)	13.4(0.8)	5.1(1.0)	11.9(0.5)
Pa_{CO_2} (kPa)	5.8(0.4)	7.8(2.3)	5.7(0.1)
A-aDO_2 (kPa)	0.7(0.2)	7.1(3.0)	2.9(0.4)

Data are expressed as mean ± SD; $\dot{mVi}$ = mean inspiratory airflow; $\dot{mVe}$ = mean expiratory airflow; Pplmin = the lowest Ppl during inspiration; $\dot{W}$vis = viscous work of breathing; HR = heart rate.

Results

Thirty minutes after their first 3-MI treatment, all calves showed dullness, anorexia, ataxia, ptyalism, weeping, increasing frequency of urination and defecation, and kicking motions of the feet to the abdomen. Ruminal motility was never affected. These early clinical signs gradually disappeared after a few hours and were followed by the onset of respiratory problems.

Three calves (No. 1, 2, and 3) developed acute RDS, showing large increases in f, $\dot{V}e$, maxdPpl, $\dot{W}$vis, and Pa_{CO_2} and large decreases in V_t, Cdyn, and Pa_{O_2}. Total pulmonary resistance and t_I/t_{TOT} did not change in any of the calves (Table 2). They died at 36, 38, and 84 hours after the 1st dosing, respectively, and were necropsied within 6 hours. The lungs were heavy, due to severe alveolar and interstitial edema. Interstitial emphysema was another prominent feature. The trachea and bronchi contained some frothy fluid. Moreover, acute thrombosis of the left pulmonary artery was seen in calf 1. Microscopically, the lungs had hyperemia and alveolar edema with inspissation of protein, and eventually, formation of hyaline membranes. The alveolar septa showed slight-to-moderate hypercellularity, due to mononuclear cells and neutrophilic and eosinophilic granulocytes. In the alveoli, slight-to-moderate numbers of macrophages and some neutrophilic and eosinophilic granulocytes were present. In several terminal bronchioles, focal necrosis of the epithelium or denudation of the basement membrane was seen. In the animal which died after 84 hours, hyaline membranes were not pronounced. Marked alveolar epithelial hyperplasia was present in several lobules.

Calves 4 and 5 showed less severe signs of respiratory tract disease after the 1st 3-MI treatment, with a moderate increase of f, $\dot{V}e$, and $\dot{W}$vis and a moderate decrease of V_t, Cdyn, and Pa_{O_2} (Table 2). Plasma 3-MI concentrations at 6 hours after the 1st and last dosings are given in Table 1. The dyspnea and tachypnea disappeared approximately 48 hours after dosing. Pulmonary function values 24 hours after the 1st dosing are given in Table 2. Comparison of means before and after the 1st 3-MI dosing was performed only for values which showed an equality of variance of the data (F test; $P < 0.05$) (ie, t_I/t_{TOT}, Pplmax, V_t, and R_L). In these 4 values, only Pplmax and V_t were significantly different before and after treatment (Student's t test for paired data; $P < 0.05$). After the 2nd 3-MI treatment, calf 4 progressively developed acute RDS with clinical and radiologic signs of pulmonary edema, emphysema, and unilateral pneumothorax. Pulmonary function values of this calf then were similar to those recorded in calves 1, 2, and 3 after the 1st dosing except for Ppl_{fre}, which was less negative and Cdyn which showed a smaller decrease.

Calf 4 survived the 2nd and the 3rd 3-MI dosings, showing a gradual amelioration of clinical and physiologic signs, a resolution of the pneumothorax, and progressively less reactivity to 3-MI treatments. Effects of 3-MI on calf 5 became gradually less. Pulmonary function values recorded after the 5th dosing did not differ from values predicted for this animal.[10]

Lung biopsy materials from calves 4 and 5 revealed no abnormalities, which finding agrees with the findings of Logan et al[3] who reported that animals became tolerant to oral 3-MI administration.

Discussion

Individual variations of the pneumotoxicosis induced by oral 3-MI administration and progressive tolerance to 3-MI treatment, as observed in 2 calves from the present experiment, have also been reported in adult cattle.[1,3]

However, clinical signs of respiratory distress were observed sooner, and pulmonary toxicosis was more severe in the present calves fed hay and concentrate than in calves from another study which were fed milk replacers.[4] It, therefore, seems likely that the severity of 3-MI pneumotoxicosis depends not only on the initial dosing, route of drug administration, and animal's tolerance and age, but also on the feeding of the animal. Study was not made of the correlation between the biological and biochemical rumen composition and the 3-MI toxicosis.

Pulmonary function changes by acute RDS have not yet been reported in calves. The ventilatory failure (hypoxemia, hypercapnia, and acidosis) was so severe in calves 1, 2, and 3, that it seemed to reflect both a decrease of alveolar ventilation and an increase of ventilation-perfusion inequalities, as indicated by the decrease of V_t and by the increase of A-aDO_2, respectively. The limits of this last measurement in pathologic conditions have been discussed.[12] Alveolar lesions (ie, edema, hyaline membrane, and epithelialization observed microscopically) also indicate a diffusion impairment existed.

Acute RDS did not increase R_L in the calves of the present study. This was consistent with our pathologic examination which showed only minimal lesions in the cranial and caudal portions of the airways. Therefore, the decrease of Cdyn, not associated with an increase of R_L, is probably mainly due to the diminution of lung volume by the alveolar flooding and to the disturbance of elastic properties of the lung by interstitial edema.[12]

The cause of the rapid shallow breathing in the present calves given 3-MI was not determined. Interestingly, in the present study, there was significant correlation ($r = -0.93$; $P \leq 0.01$) between Pa_{O_2} and f, indicating that tachypnea may be partially caused by stimulation of aortic body chemoreceptors. However, it would be useful to measure the effect of bilateral vagotomy in treated calves. It has been shown in the pony that tachypnea induced by 3-MI was due to stimulation of pulmonary afferent-receptors systems.[13] Reduction of V_t and simultaneous increase of f observed in the present calves have also been described during acute RDS in human newborns,[14,15] in which the theory of the minimal ventilatory work was found to be a possible explanation.[16,17] The increase of $\dot{V}_{vis}$ in calves showing acute RDS could indicate that energetic cost of breathing may be so high in this disease, that death would be due to the increasing ventilatory failure resulting from a progressive exhaustion.

Bronchiolar lesions described in the present study are not usually reported in instances of 3-MI pneumotoxicosis. However, similar lesions in cattle,[1] sheep,[18] and ponies[13] have been reported.

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