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Low Dose Monocrotaline Causes a Selective Pulmonary Vascular Lesion in Male and Female Pneumonectomized Rats

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Abstract

Purpose/Aim: Low doses (30–80 mg/kg) of monocrotaline are commonly used to create experimental models of pulmonary hypertension in rats. At these doses, monocrotaline causes pulmonary endothelial apoptosis and acute lung injury which ultimately resolves into pulmonary vascular disease. Higher doses of monocrotaline (300 mg/kg) are known to create severe liver injury, but previous investigations with lower doses have not reported histology in other organs to determine whether the vascular injury with monocrotaline is pulmonary-selective or generalized.

Materials and Methods: We therefore sought to determine whether monocrotaline caused extra-pulmonary injury at doses commonly used in pulmonary hypertension studies. We performed left pneumonectomy on young male and female rats before administering 50–60 mg/kg monocrotaline 7 days later. We monitored serum chemistry and urine dipsticks during the first 3 weeks while the animals developed pulmonary hypertension. After 3 weeks, we sacrificed animals and stained the lungs and highly vascular visceral organs (kidney, liver, and spleen) for elastin to evaluate the degree of vascular injury and remodeling.

Results: We did not observe proteinuria or significant transaminitis over the 3 weeks following monocrotaline. As previously published, monocrotaline caused severe pulmonary vascular disease with neointimal lesions and medial hypertrophy. We did not identify significant large or small arterial damage in the kidneys, liver, or spleen. Two external veterinary pathologists did not identify histopathology in the kidneys, liver, or spleen of these rats.

Conclusions: We conclude that 50–60 mg/kg of monocrotaline causes a selective pulmonary vascular lesion and that male and female rats have little non-pulmonary damage over 3 weeks at these doses of monocrotaline.

Keywords

monocrotaline; pulmonary hypertension; endothelium; kidney; liver

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Declaration of Interests:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Introduction

Pulmonary arterial hypertension (PAH) is a devastating, progressive disease characterized by increased precapillary resistance in the pulmonary circulation (1). Although the pathogenesis is poorly understood, human pathology studies demonstrate disorganized endothelial proliferation, inflammation, thrombosis, and medial hypertrophy (2). The combination of pulmonary vascular injury and increased vasoconstriction reduces pulmonary blood flow causing right ventricular dysfunction and death (3). Current treatments improve exercise tolerance and probably improve survival, but median survival is still only seven years, and novel treatment approaches are desperately needed (4).

Multiple animal models including mice, rats, dogs, and pigs have been developed trying to replicate the human disease (5). We have reported a reproducible and consistent model of severe PAH obtained by injecting Sprague-Dawley rats with low dose monocrotaline (MCT), 50–60 mg/kg one week following pneumonectomy (6). The MCT and pneumonectomy model reproduces key features of human pathology with neointimal formation, smooth muscle hypertrophy, and proliferative lesions. It causes severe hemodynamic PH and progressive right heart failure by week 4. It also causes equally severe PH in males and females, a significant advantage for a disease that most often afflicts females. In contrast, MCT treatment alone (a commonly used PH model) generally causes relatively isolated smooth muscle hypertrophy without the more proliferative lesions observed with pneumonectomy + MCT; most all reports with MCT alone model are in male rats with females being disease resistant. Because of the severe and reproducible phenotype, the pneumonectomy + MCT model is appealing to understand mechanisms of disease progression and test new treatment strategies.

MCT is a pyrrolizidine alkaloid derived from the seeds of *Crotalaria spectabilis* (7, 8), and was first described to cause rodent PH in 1961 (9). At low doses, the native MCT molecule does not injure cultured endothelial cells (10). MCT is metabolized within the liver via the cytochrome P-450 3A4 isoenzyme to produce the toxic pyrrole metabolite dehydromonocrotaline (MCTP)(7, 11). Males have a more active P450–3A metabolism which explains the increased male susceptibility to the toxic effects of MCT and subsequent development of PH (7, 8). MCTP has a short half-life of 3 seconds before degradation occurs in plasma (12). Following MCT injection, pyrrolic metabolites have been identified in liver, lung, and kidney tissue (13). After metabolic activation in the liver, MCT causes a variety of toxic insults that vary by dosage (45–300 mg/kg) and include pulmonary endothelial apoptosis, acute lung injury, pulmonary fibrosis, necrotizing pulmonary arteritis, myocarditis, hepatic venoocclusive disease, pulmonary hypertension, and right ventricular hypertrophy (14, 15). Two weeks after male Sprague-Dawley rats were exposed to 60 mg/kg of MCT, Lee demonstrated that injury was isolated to the arterial vasculature with lung parenchyma, vein, and bronchioles being spared; at 5 weeks, the veins and bronchioles remained normal (16). The mechanism by which MCTP causes PH is not completely understood (5, 15, 17). In endothelial cell culture, MCTP causes DNA crosslinking and therefore inhibits cell proliferation by interfering with cell cycle progression (18–20). MCTP also covalently binds a variety of cellular proteins causing partial or total loss of function and leading to apoptosis (10, 17). Cytotoxicity in bovine pulmonary artery endothelial cells

develops within 48 hours of being exposed to MCTP, with decreased proliferation, increased hypertrophy, and decreased synthetic capabilities (10). In the pneumonectomy/MCT model that we have previously reported with a 60 mg/kg dose, proliferative vascular changes are seen as early as 1 week after MCT injection and fully developed pathologic changes occur by day 21 (6); other investigators using MCT injection without pneumonectomy have reported initial mild subintimal changes followed by a proliferative vasculitis with smooth muscle hypertrophy and pulmonary hypertension developing in 1–2 weeks after injection (5, 15).

There has not been a systematic evaluation of other vascular visceral organ (kidney, liver, and spleen) function and histology following a single dose of MCT injection while PH develops. At least one report by Stewart has documented a late kidney pathology using the MCT model without pneumonectomy (21), but that study did not systematically address earlier time points nor assess other organs. The MCT/pneumonectomy model might be less attractive as a drug development tool if there were consistent evidence for liver or kidney injury due to MCT injection. To address this gap, we assessed hepatic and renal function, urinalysis, and histology at different time intervals after we administered MCT to Sprague-Dawley rats and evaluated specifically for pathologic evidence of vascular injury in these organs.

Methods

Animals

All animal studies were approved by the Institutional Animal Care and Use Committee at the University of Rochester before initiation. Young rats were allowed food (normal lab animal diet provided by the vivarium) and water *ad libitum*. They were housed no more than 3 to a cage on corn cob bedding. The animals were maintained on a 12-hour light/dark cycle in a controlled temperature of 74 ° F with humidity of 37% environment. Procedures were performed after 8 weeks of age when their weights were >200 g.

Treatment Protocol

We made our initial histopathologic observations in post-mortem liver, kidney, and spleen from animals that were sacrificed for other experiments at day 21 using the 60 mg/kg MCT dose which the lab had been using for several years (Table 1, Groups 4 and 5). Subsequently, across all of the lab's experiments, we reduced the severity of the model by reducing the MCT dose to 50 mg/kg. We therefore performed a time course with metabolic carts for urine and serial blood draws using this 50 mg/kg dose. We studied 6 females and 6 males at 50 mg/kg MCT following pneumonectomy (Table 1, Group 3). We then studied 3 females and 3 males to evaluate for the effect of anesthesia and pneumonectomy (without MCT, Table 1, Group 2). Finally, we studied 4 un-manipulated males (Table 1, Group 1) and finding nothing, we didn't separately sacrifice un-manipulated females. For animals undergoing pneumonectomy, we vaporized isoflurane at 4% to induce anesthesia and maintained a surgical plane of anesthesia at 2% before doing left pneumonectomy as previously described (6). Rats were exposed to isoflurane for less than 30 minutes, usually 15 minutes. Rats recovered in the vivarium and received either 50 or 60 mg/kg MCT (Sigma-Aldrich (St.

Louis, MO)) intraperitoneal injection seven days later; these were the doses we and others were commonly using to induce PH. An additional group of historical rats did not have a pneumonectomy and received only a single 60 mg/kg injection to evaluate whether pneumonectomy itself changed extra-pulmonary MCT toxicity.

Histology

At sacrifice, we anesthetized rats with isoflurane 4% before utilizing an 18-gauge catheter to perform tracheostomy. Mechanical ventilation was used to support rats at 90 breaths/min with a 2 ml tidal volume. We opened the abdomen and removed the liver, spleen, and kidneys into a solution of neutral buffered formalin. The specimens were processed and paraffin embedded. We sent paraffin embedded tissue to an independent veterinary lab, IDEXX BioResearch Laboratory in Columbia, Missouri, where two blinded pathologists assessed for cellular damage caused by MCT. The pathologists requested four normal male rats to serve as controls to increase the integrity of the data and account for our vivarium conditions and any stresses the animals might experience. They made their own slides using hematoxylin and eosin stain, and we made separate slides with a modified collagen and elastin stain as previously described (6). The pathologists reviewed the slides independently. Inter-observer agreement could not be calculated because they did not provide us the information on a slide-by-slide basis but rather in aggregate. A second pathologist reviewed the slides independently and verbalized agreement with the first. For animals injected with MCT, we sacrificed a group at day 8 post MCT injection and another group between day 18–24 post MCT. The pneumonectomy alone group (no MCT) was studied day 15 post pneumonectomy (day 8 post MCT injection and day 15 post pneumonectomy are the same time point after surgery).

Blood and Urine Sampling

To evaluate liver and renal function with and without MCT, routine blood chemistries and urine were collected from 18 rats, nine males and nine females, at different time points (Table 1, Groups 2 and 3). The rats were sedated with isoflurane, less than 5 minutes, before a jugular stick was performed. We collected 1 ml of blood into a lithium heparin tube on ice. We spun the tube for 10 minutes at 2,000 RPM on an Eppendorf 5804R centrifuge. Veterinary Lab of Rochester completed an independent analysis. We collected urine from individual rats in a metabolic cage until 0.5–1 ml of urine was obtained. The urine was evaluated with Siemens Multistick 10 SG dipstick.

Results

For the 18 rats deliberately assigned a treatment in Table 1, groups 2 & 3, the planned measurements were collected on all of the rats without death or data loss. We found no evidence for direct MCT toxicity in liver, kidney, or spleen at day 8 or day 18–24 post injection (Figure 1). There was no data loss in the unmanipulated males (Group 1); the organs for Groups 4 & 5 were taken from unrelated experiments at the time we first asked the question about extra-pulmonary toxicity. Males and females treated with 50 mg/kg MCT after pneumonectomy developed very severe PH and right heart failure within 21 days, often dying by day 28 (manuscript in preparation).

Histology

In the rats without MCT, the licensed veterinary pathologists found no consistent renal abnormalities. Unmanipulated rats (Table 1, Group 1) showed scattered protein casts in all four animals with two having interstitial lymphoid infiltrates. Pneumonectomy alone rats (no MCT, Group 2) had perivascular lymphoid infiltrate (two animals) and tubular regeneration (one animal). The detailed histologic findings are summarized in Table 2.

In the MCT treated rats, the pathologists found renal changes similar to the two control groups. There was no evidence of direct toxicity from MCT or injury from chronic pulmonary hypertension in rats at day 8 or day 18–24 post-MCT injection. Group 3 had one female with perivascular lymphoid aggregates. Group 4 and 5 had four rats with scattered protein casts. Overall, there was no difference in kidney histology between the untreated and the MCT treated groups.

In the rats without MCT, the pathologists found no liver injury. Group 1 had no abnormal findings. Group 2 (pneumonectomy alone) had four with few microgranulomas and four with foci of erythropoiesis, which may be an interesting finding after pneumonectomy.

In the rats treated with MCT the pathologists found no consistent injury in rats sacrificed at day 8 (Group 3, early). Four rats from the early group had foci of erythropoiesis and three with microgranulomas, similar to the findings of the pneumonectomy alone rats (Group 2). As expected, all of the day 18–24 rats (Group 3, late) had hepatocyte atrophy, fibrosis, and necrosis, consistent with passive hepatic congestion (due to chronic pulmonary hypertension and right heart failure). In Group 4 (MCT alone), four rats had microgranulomas and one had atrophied hepatocytes around the central vein with congested and dilated sinusoids. These findings are likely consistent with the less severe pulmonary hypertension and right heart failure which is observed 3 weeks after MCT alone. Group 5 (pneumonectomy and 60 mg/kg MCT) had the most abnormal findings including atrophied hepatocytes around the central vein with congested and dilated sinusoids in three males and all five females. One male had focal portal neutrophilic infiltrate, one female had mild neutrophil and lymphocyte infiltrate with fibrosis and random subcapsular necrosis, and one female had centrilobular hepatocellular apoptosis and necrosis. These findings likely represent the more severe degree of right heart failure which we observe in the pneumonectomy + 60 mg/kg MCT as compared to pneumonectomy + 50 mg/kg MCT.

All of the splenic samples were normal in both MCT treated and untreated rats without lesions in the red pulp or the vasculature.

Serum Chemistry

We observed no change in ALT, total bilirubin, GGT, cholesterol, triglyceride, or albumin in groups 2 and 3 (Table 3). There was isolated, low-grade elevation of AST in both the male and female rats from group 2 (pneumonectomy alone, data not shown) two weeks after surgery. In all groups there was consistent hyperglycemia due to the stress of manipulation and phlebotomy; this is a well-recognized phenomena after rat blood draws. The only elevation of ALT occurred in the group 3 males at day 21 post injection at a time when right

heart failure was expected; congestive hepatopathy was observed on histology. We did not evaluate the females at this later time point.

Electrolytes were normal in all the groups (data not shown). The creatinine did not differ between groups and remained normal despite the development of pulmonary hypertension. The BUN remained stable at initial time points after MCT but ultimately rose parallel to the development of right heart failure (day 21). The normal renal function and electrolytes are congruent with the normal histologic findings.

Urine Analysis

Finally, we assessed urine by dipstick. In group 2, the females developed new 'large' blood on day 15 post pneumonectomy in the absence of bilirubin, urobilogen, or significant proteinuria (data not shown). They also had normal electrolytes, BUN, and creatinine (as above). Histology the following day was normal. Microscopic hematuria was not observed in the MCT treated females at day 8 (Table 4), and we did not do urinalysis in the females at day 14 or day 21. The males from group 2 also developed new moderate blood (data not shown) despite having normal creatinine, electrolytes, and histology the following day. Myoglobin may have cross reacted with the hemoglobin measure on the dipstick as a potential explanation unrelated to blood, but it is odd that males treated with MCT (Table 4) did not have substantial hemoglobin (similar to the day 8 MCT treated females). Similarly, males and females with pneumonectomy alone leaked protein (data not shown, males at day 15 more so than other groups), but males and females treated with MCT (and pneumonectomy) did not leak substantial protein.

Discussion

The MCT model of pulmonary hypertension has been utilized for decades (5), and more recently, we have utilized MCT seven days after pneumonectomy to produce a more consistent model of severe pulmonary hypertension with advanced right heart failure and pathologic lesions that reflect human disease(6). We observe these changes at lower MCT doses relative to reports from groups doing MCT alone who have observed some neointimal formation (22). The present study is the first to evaluate rats carefully for liver, kidney, and spleen injury at doses of MCT commonly used to cause PH. Our comprehensive analysis of urine, blood, and histology at different time points strongly supports the conclusion that MCT-mediated vascular injury is isolated to the lung (at these doses). The only consistent abnormal finding we observed was hepatic congestion with elevated transaminases after overt right heart failure had developed. Specifically, there was no evidence for splenic, liver, or kidney injury at one week following MCT in either sex, and there was no evidence for histologic kidney disease even after pulmonary hypertension and right heart failure were clearly established. The degree of hepatic congestion observed on histology was more severe in the pneumonectomy + MCT 60 mg/kg treated rats as compared to the 50 mg/kg. This correlates with more severe right ventricular dysfunction and more severe pulmonary hypertension, despite a relatively modest increase in MCT (manuscript in preparation). This appears to be an exciting area for further investigation with such a steep MCT dose response in the pulmonary vascular injury and repair. Our findings suggest that toxicity with low dose

MCT, 50–60 mg/kg, in both male and female Sprague-Dawley rats is limited to the pulmonary vasculature with subsequent hepatic injury occurring as a result of right heart failure.

Extra-pulmonary endothelial injury occurring after MCT has been reported. Roth evaluated the effects of MCT ingestion on liver, kidneys, and lungs after administering MCT (22 µg/ml) to young male rats in the drinking water for 28 days with injury occurring in all three organs (23). It is difficult to know the amount of MCT the rats ingested, and their experiments created relatively continuous exposure over weeks, very different than the typical single injection used to create experimental PH. At day 21 after continuous MCT treatment in the drinking water, there was evidence of right ventricular hypertrophy (23).

Intraperitoneal injections of 300 mg/kg MCT in Sprague-Dawley rats have resulted in hepatotoxicity occurring as short as 8 hours after injection. Death occurred in some animals within 24 hours, and histologic findings included coagulative and single cell necrosis, necrotic parenchymal cells in the periportal regions, hemorrhage and widening of the sinusoids in the centrilobular region, apoptosis both within and outside of the centrilobular lesions, and inflammatory infiltrate present within the centrilobular lesions (24). At 160 mg/kg given through gavage, hepatic venoocclusive disease was observed to occur with elevation of bilirubin in less than 10 days after ingestion; milder disease occurred at 100 mg/kg (25). In both of these studies, the doses used to induce hepatic injury were much greater than those typically used to create experimental PH, hepatic injury appeared to occur as a primary event, and authors did not comment on the pulmonary vasculature.

In contrast, our histology, blood chemistry, and urinalysis were all normal at 1–2 weeks after MCT injection, and our liver abnormalities only occurred after right heart failure developed with 9/14 rats showing signs of hepatic congestion between day 18–24 post MCT injection. This late time point is well outside of the time frame of the above studies in which MCT caused direct hepatic injury. With regard to the confound of anesthesia in our model, higher isoflurane doses, longer duration of exposure, and low oxygen content are needed to cause hepatic injury from isoflurane exposure (26).

Many investigators have attempted to generate severe PH in mice. A mouse PH model is appealing given the potential for genetic engineering and pathway isolation, but unfortunately, investigators have not had success creating PH at a single intravenous low dose of MCTP, 8–15 mg/kg (27). Older literature evaluated a variety of daily oral doses and found that mice were extremely resistant to the development of PH and right heart failure even after daily oral ingestion of MCT at 24 mg/kg (28). Weekly subcutaneous doses of MCT at 100–200 mg/kg produced fatal pulmonary fibrosis (29). More recently, higher doses of 500 mg/kg given twice (one week apart) (30) and 600 mg/kg weekly for four weeks have been used to cause PH in mice (31). At these high doses a selective pulmonary vascular lesion seems quite unlikely as Yao and colleagues recently demonstrated severe liver injury and moderate kidney injury occurring within 24 hours after a single 500 mg/kg dose of intraperitoneal MCT (32). Although this may be strain dependent, the bulk of the evidence in mice and rats suggest that higher doses of MCT are hepatotoxic, and thus ‘experimental PH’ results in mice at these high doses must be interpreted with caution.

MCT mediated kidney injury has also been reported. Carstens fed Sprague-Dawley rats chow with low dose MCT (Crotalaria seed concentration of 0.02–0.08% that increased over time), for eight months and then observed glomerular hyalinization and arterial thickening with luminal narrowing (33); we did not observe similar findings with our single MCT exposure. Similar to the Roth study above, it is hard to know how much MCT the rats ingested and whether daily exposure produces a different kind of injury through chronic glutathione depletion. Kurozumi found progressive renal injury starting two weeks after injecting 50 mg/kg MCT intraperitoneally in Wistar rats (34); serum creatinine increased from normal during the first two weeks to 1.8 mg/dl on week 3 and up to 4.22 mg/dl at week 4 (34). They observed progressively severe histopathologic changes. Using 75 mg/kg MCT in a Fisher-344 rat model of experimental PH, Stewart and colleagues observed gross renal enlargement with microscopic glomerular enlargement, loss of mesangial cells, and tubule hyalinization (21). These authors postulated that the renal pathology was a long term consequence of MCT-mediated injury, but the interpretation is complicated by the administration of genetically modified endothelial progenitor cells as PH therapy 21 days after MCT injection (21). Moreover, Stewart's report describes late injury while Kurozumi described injury occurring as early as 3 weeks post injection. In Stewart's model only the treated rats survived long enough (7 weeks) to manifest renal injury. In contrast, we found no evidence for renal injury 21 days after MCT in our model even when the animals had severe right heart failure. It is difficult to postulate that our rats would have developed advanced microscopic changes of kidney damage related to MCT over the next 4 weeks without any microscopic histologic changes (and essentially normal creatinine and urinalysis). It does seem conceivable that 75 mg/kg is nephrotoxic, and our experiments do not exclude that possibility. Another possibility is that Fisher and Wistar rats are more susceptible to MCT mediated renal injury (we used Sprague-Dawley rats). Glutathione modulates the sensitivity of the renal tissue to the active pyrrole metabolite (13), and it is conceivable that differences in glutathione concentrations explain the difference in susceptibility to renal injury between the two strains. In three-month old Fisher rats without injury, the Glutathione content in the liver was 22 nmol/mg, 8 nmol/mg in the kidney, and 6 nmol/mg in the lung, and all three levels were found to naturally decrease with age (35). Comparatively, male Sprague-Dawley rats between 200–250 g without injury had much higher glutathione levels of 97 nmol/mg in the liver, 60.4 nmol/mg in the kidney, and 42.5 nmol/mg in the lungs (13). After MCT injection, glutathione levels have been shown to increase in the renal tissue of male Sprague-Dawley rats (13) which could provide more renal protection from MCT injury. We hypothesize that the Spragues are more resistant to renal injury than Wistar or Fisher because of the higher renal glutathione content.

Animal models of PH offer the possibility to study preclinical drug development in a fashion that meaningfully advances a drug towards human clinical trials. Although previous studies generally focused on isolated mechanisms of action, there is emerging consensus that future late-stage preclinical studies should utilize multiple animal models and include currently-approved human therapies along with the novel drug (pre-clinical combination therapy) (36). In light of the present data showing relatively selective pulmonary vascular damage, we propose that pneumonectomy-MCT is a robust model for preclinical drug development.

In conclusion, the present study is the first to demonstrate that MCT does not directly cause vascular lesions in the liver, kidney, and spleen of rats during the time frame in which pulmonary hypertension initially develops. Our thorough, longitudinal analysis demonstrates that low dose MCT is selective in causing pulmonary vasculature injury in both sexes; extra-pulmonary hepatic injury is due to chronic pulmonary hypertension with right heart failure. This selectivity is apparently lost with repeated and/or higher dose exposures in rats. Mice do not express sufficient levels of the 3A4 isoform of cytochrome P450, and thus do not make sufficient quantities of the active ppyrole metabolite to create a selective pulmonary vascular lesion. We would caution against the use of high dose, repeated exposures of MCT as a model of PH in mice until investigators demonstrate that this causes a selective pulmonary vascular lesion. All of the available data in rats and mice suggests that there would be substantial hepatic and renal toxicity with such exposures.

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Figure 1.

Histology from normal rats and rats treated with MCT. **A.** H&E stained photomicrograph showing normal hepatocyte and liver architecture of normal rat. **B, C.** Representative H&E stained photomicrographs showing normal hepatocytes of a female (**B**) and male (**C**) rat 8 days after 50 mg/kg MCT injection; there are no areas of necrosis. **D.** Representative H&E stained photomicrograph showing hepatic parenchyma with sinusoidal disruption adjacent to central vein consistent with hepatic congestion 24 days after 60 mg/kg MCT injection. **E.** Representative H&E stain photomicrograph showing normal glomeruli and tubules in a female rat 8 days after 50 mg/kg MCT injection. **F.** Representative CME stained photomicrograph of the spleen showing normal arteriole 8 days after 50 mg/kg MCT injection.

Table 1.

Group designations for evaluation of extra-pulmonary toxicity after injection with monocrotaline.

36 Sprague Dawley Rats (22 Males and 14 Females)	Labs Day 7	Labs Day 14	Early Histology	Late Histology
Group 1: 4 Un-manipulated males.				X
Group 2: 3 males and 3 females underwent left pneumonectomy only (No MCT).	X	X	X	
Group 3: 6 males and 6 females underwent pneumonectomy followed by MCT 50 mg/kg intraperitoneal injection 1 week later; (3 of each sex sacrificed for early histology, 3 sacrificed day 21 for late histology)	X	X [*]	X	X
Group 4: 5 males received 60 mg/kg MCT intraperitoneal injection (no pneumonectomy).				X
Group 5: 4 males and 5 females underwent pneumonectomy followed by 60 mg/kg MCT intraperitoneal injection 1 week later.				X

*
n=3 males only, labs at both day 14 and 21

Table 2

Histologic Findings.

Specimen-Diagnosis	Group 1	Group 2	Group 3	Group 4	Group5
Kidney- Few foci of tubular regeneration (0–10 foci)	2/4	1/6	0/12	5/5	4/9
Kidney-Focal interstitial lymphoid infiltrates	2/4	0/6	0/12	0/5	0/9
Kidney- Focal perivascular lymphoid infiltrate	0/4	2/6	1/12	0/5	0/9
Kidney- Protein casts (0–10 tubules)	4/4	0/6	0/12	4/5	4/9
Kidney- Tubular mineralization (0–10)	0/4	1/6	1/12	2/5	1/9
Liver- Few scattered foci of erythropoiesis	0/4	4/6	4/12	0/5	0/9
Liver-Focal portal lymphoid infiltrate	0/4	1/6	0/12	0/5	1/9
Liver-Focal portal neutrophilic infiltrate	0/4	0/6	0/12	0/5	1/9
Liver-Hepatocyte atrophy	0/4	0/6	0/12	1/5	8/9
Liver-Microgranulomas	0/4	4/6	3/12	4/5	4/9
Liver-Necrosis	0/4	0/6	0/12	0/5	1/9
Liver-Necrosis and apoptosis	0/4	0/6	0/12	0/5	1/9
Liver-Portal hyperplasia	0/4	0/6	0/12	0/5	1/9
Liver-Subcapsular Necrosis	0/4	0/6	0/12	0/5	1/9
Splenic lesions	0/4	0/6	0/12	0/5	0/9

Table 3.

Chemistry from pneumonectomy + MCT treated rats.

	Pneumonectomy Plus 50 mg/kg Monocrotaline						
	Female		Male				p-value
	<u>Day 0</u> <u>(Prior to</u> <u>MCT</u> <u>injection)</u>	<u>Day 7 Post</u> <u>MCT</u> <u>Injection</u>	<u>Day 0 (Prior</u> <u>to MCT</u> <u>injection)</u>	<u>Day 7 Post</u> <u>MCT</u> <u>Injection</u>	<u>Day 14 Post</u> <u>MCT</u> <u>Injection</u>	<u>Day 21 Post</u> <u>MCT</u> <u>Injection</u>	
Cholesterol (mg/dl)	65 ± 7	66 ± 9	65 ± 6	80 ± 17	71 ± 9	65 ± 12	0.02
Triglyceride (mg/dl)	78 ± 49	48 ± 26	108 ± 44	49 ± 8	86 ± 17	49 ± 21	0.03
GGt (IU/L)	0.17 ± 0.4	0.17 ± 0.4	0 ± 0	0.33 ± 0.8	0 ± 0	0 ± 0	0.95
ALT (U/L) (20–92)	50 ± 9	37 ± 9	52 ± 9	59 ± 10	57 ± 4	227 ± 279	0.007
AST (U/L)	83 ± 47	68 ± 6	75 ± 3	73 ± 14	69 ± 8	243 ± 239	0.047
Total Bilirubin (mg/dl)	0.5 ± 0.25	0.13 ± 0.1	0.13 ± 0.12	0.3 ± 0.2	0.17 ± 0.12	0.17 ± 0.06	0.10
Alkaline Phosphatase (U/L) (16–96)	199 ± 71	186 ± 93	367 ± 158	402 ± 197	541 ± 201	270 ± 91	0.01
Creatinine (mg/dl) (0.2–0.8)	0.4 ± 0.1	0.37 ± 0.12	0.52 ± 0.19	0.27 ± 0.1	0.3 ± 0	0.5 ± 0.3	0.35
Glucose (mg/dL) (50–135)	209 ± 38	204 ± 23	240 ± 21	226 ± 8	203 ± 21	169 ± 30	0.52
BUN	17 ± 2.2	15 ± 4.2	16 ± 2.2	17 ± 1.9	18 ± 0.7	30 ± 16	0.004
Albumin (g/dl)	2.7 ± 0.1	2.5 ± 0.3	2.5 ± 0.1	2.6 ± 0.2	2.8 ± 0.1	2.5 ± 0.2	0.03

Table 4.

Urine analysis from pneumonectomy + MCT treated rats.

	Pneumonectomy Plus 50 mg/kg Monocrotaline					
	Female		Male			
Urine Analysis	Day 0 (Prior to MCT injection)	Day 7 Post MCT Injection	Day 0 (Prior to MCT injection)	Day 7 Post MCT Injection	Day 14 Post MCT Injection	Day 21 Post MCT Injection
Leukocytes	Negative	Negative	Negative	Negative	Negative	Negative
Nitrite	Negative	Negative	Negative	Negative	Negative	Negative
Urobilogen	Negative	Negative	Negative	Negative	Negative	Negative
Protein	1 ± 0	1 ± 0.6	1.5 ± 0.5	1.7 ± 0.8	2.3 ± 0.6	3 ± 1
pH	7.3 ± 0.25	7.5 ± 0.6	7.3 ± 0.25	7.4 ± 0.5	7.7 ± 0.3	6.8 ± 0.8
Blood	Negative	Negative	Negative	Mild	Negative	Mild
SG	1.016 ± 0.01	1.01 ± 0.01	1.0125 ± 0.01	1.009 ± 0.04	1.01 ± 0.005	1.018 ± 0.01
Ketones	Negative	Negative	Negative	Negative	Negative	Negative
Bilirubin	Negative	Negative	Negative	Negative	Negative	Negative
Glucose	Negative	Negative	Negative	Negative	Negative	Negative

Urine dipstick blood grading scale: Negative – 0, Trace – 1+, Mild – 2+, Moderate – 3+, Large – 4+