

Early Inflammatory Response of Minocycline and Tetracycline on the Rabbit Pleura*

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The histopathologic findings were compared from 20 mg/kg intrapleural tetracycline hydrochloride (TCN) and three doses of intrapleural minocycline hydrochloride (5, 10, and 20 mg/kg) (MCN) in New Zealand white rabbits. Both TCN and MCN produced an early neutrophilic predominant pleural effusion that became mononuclear over 48 h. There was no difference in pleural fluid accumulation, number of adhesions, or histologically measured visceral and parietal pleural thickness between TCN and MCN (all $p=ns$). The TCN, 20 mg/kg, produced more visceral pleural plaque than MCN, 5 mg/kg ($p<0.05$).

Symptomatic pleural effusions occur frequently in patients with malignancy.¹ Because patients with malignant pleural disease can survive for several months to years, palliative treatment with low morbidity and cost are important in their management. Although thoracentesis or tube thoracostomy can transiently relieve dyspnea, chemical-induced pleural inflammation is commonly used to produce pleurodesis and prevent fluid reaccumulation.^{2,3}

Tetracycline hydrochloride (TCN) has proven to be an effective, inexpensive pleurodesis agent with minimal morbidity.^{4,6} The recent pharmaceutical decision to withdraw tetracycline has created a problem in the medical community to find cost-effective alternative therapies. While various agents have been utilized, including bleomycin,⁷ quinacrine,⁸ talc,⁹ 5-fluorouracil,¹⁰ thiotepa,¹¹ and radioisotopes,¹ TCN compares favorably to these in terms of efficacy, cost, safety, and ease of administration.

Recent studies have suggested that other tetracyclines, including minocycline (MCN), may be efficacious in the treatment of malignant pleural effusion.¹²⁻¹⁴ Preliminary work suggests that intrapleural MCN and TCN produce a similar degree of pleural symphysis in a rabbit model.¹⁵ Using that model, we compared the pleural histologic condition and cellular response after intrapleural instillation of 20 mg/kg TCN and 5, 10, and 20 mg/kg MCN.

METHODS

Forty-eight New Zealand white rabbits weighing 2 to 3 kg were lightly anesthetized with 80 to 100 mg of ketamine hydrochloride (Ketalar, Parke-Davis, Morris Plains, NJ) and 0.25 mg/kg acepro-

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Increasing MCN doses resulted in greater pleural fluid neutrophil accumulation. With higher dose MCN, greater mesothelial cell desquamation and fibroblast proliferation was evident compared to the 5 mg/kg dose. The MCN and TCN produce similar histopathologic condition in the rabbit pleura which suggests that MCN should cause a similar clinical response in humans. (*Chest* 1993; 104:1585-88)

MCN = minocycline hydrochloride; TCN = tetracycline hydrochloride

mazine (PromAce, Aveco, Fort Dodge, Iowa) via the lateral ear vein. The right chest wall was shaved, and a 1.0-cm skin incision was made midway between the spine and scapular tip. With aseptic technique, an 18-gauge plastic catheter (Cathlon IV Striped, Critikon, Tampa, Fla) was placed percutaneously into the right pleural space.

Rabbits received intrapleural injection of 20 mg/kg TCN or 5, 10, or 20 mg/kg of MCN in 3-ml 0.9 percent NaCl (Lederle Laboratories, Pearl River, NY). Immediately after instillation, the catheter was removed. Postoperative administration of a single dose of 0.04 mg/kg subcutaneous buprenorphine (Norwich Eaton, Norwich, NY) provided narcotic analgesia for an additional 12 h.

Three milliliters of pleural fluid was obtained by thoracentesis at 24, 48, and 72 h via the fourth to fifth intercostal space ventrolaterally. Total cell count was determined by hemocytometry, and differential counts were performed after cytopspin preparation and Leukostat staining (Fisher Diagnostics, Fisher Scientific, Orangeburg, NY).

After 96 h, the rabbits were killed with intravenous pentobarbital. Using a ventral midline approach, the rabbits underwent autopsy and the thorax was resected *en toto*. All visualized pleural fluid was aspirated through a diaphragmatic incision. The right thorax was entered and pleural adhesions were counted before transection at the midline. The right hemithorax was bisected in the midaxillary plane to permit imaging in a planar view. Photographs were taken of the ventral and dorsal surfaces of the lung and the chest wall surface. The plaque area was planimetrically calculated from the parietal and visceral pleura as a percentage of total parietal and visceral pleural area, respectively on the Zeiss IBAS 2000 grid image analyzer (Zeiss Inc, Germany).

The lungs and hemithoraces were fixed in formalin, and histologic sections were prepared from the parietal pleura at the fourth intercostal space. A transverse circumferential section from the right lower lobe was obtained 1 cm from the lung base. Specimens were embedded in paraffin, cut, and stained with hematoxylin-eosin and Masson's trichrome stains. Pleural thickness was determined by the distance from the internal elastica to the free pleural space at five random high power fields and averaged for each animal. Mesothelial cell morphology and the extent of mesothelial desquamation were compared between groups.

Statistics

Data were expressed as mean \pm standard error. Analysis of variance was used to determine group differences in the amount of

pleural fluid, number of adhesions, parietal and visceral pleural percentage of fibrosis, pleural thickness, and change in pleural fluid characteristics over time. *Post hoc* testing was performed by Scheffe F-test to determine specific differences between groups. Data were computed with statistical software (Statview, SE + Graphics, Abacus Concepts, Inc.), and p values of <0.05 were considered significant.

RESULTS

Seven of the 48 rabbits did not survive to the completion of the study; autopsy of these animals confirmed intrapulmonary instillation of drug. Of the remaining, 11 were in the TCN group and 10, 8, and 12 were in the 5, 10, and 20 mg/kg MCN groups, respectively. Pleural fluid was obtained from each rabbit, with the exception of one rabbit in both the TCN and 10 mg/kg MCN groups. One rabbit from the MCN 20 mg/kg group required a 36 ml thoracentesis on day 1 due to respiratory insufficiency.

Pleural fluid cell counts at 24, 48, and 72 h are shown in Table 1. Nucleated cell counts in the 20 mg/kg MCN and TCN groups trended higher at 48 and 72 h compared to the 5 and 10 mg/kg MCN. All groups demonstrated an early neutrophil dominant effusion at 24 h. Neutrophil counts at 24 h with 5 and 10 mg/kg MCN were less when compared to 20 mg/kg TCN ($p < 0.05$). Nucleated cell counts trended higher over time in the TCN and MCN 20 mg/kg groups compared to lower dose MCN. In all groups, at 48 and 72 h, pleural fluid became more macrophage and lymphocyte predominant. Both the 5 and 10 mg/kg MCN produced a lower lymphocyte count at 72 h when compared to TCN ($p < 0.01$). No other differences between differential cell counts were observed.

Visceral pleural plaque was less with 5 mg/kg MCN compared to TCN ($p < 0.01$) (Table 2). Although TCN trended toward having more visceral plaque production, higher adhesion number, and greater mean visceral and parietal pleural width compared to all three MCN doses, no significant difference was found. Pleural fluid volume tended to be larger after 20 mg/kg TCN and MCN compared to the lower MCN dose regimens.

Fibrin strands adherent to the pleural surfaces were present in all groups. Areas of fibrin deposition were often separated by focal collections of relatively pre-

Table 1—Nucleated Cell Counts With Differentials Following Intrapleural Instillation of TCN and MCN*

	24 h	48 h	72 h
TCN, 20 mg/kg	n = 10	n = 10	n = 9
Nucleated cells $\times 10^3/\mu\text{l}$	5.0 \pm 0.7	7.9 \pm 2.2	15.0 \pm 4.3
PMN $\times 10^3/\mu\text{l}$	4.1 \pm 0.7†	3.8 \pm 1.8	2.1 \pm 0.7
M/M $\times 10^3/\mu\text{l}$	0.8 \pm 0.2	3.8 \pm 0.6	10.4 \pm 3.0
Lymphs $\times 10^3/\mu\text{l}$	0.1 \pm 0.0	0.3 \pm 0.1	2.5 \pm 0.7‡
MCN, 20 mg/kg	n = 12	n = 11	n = 11
Nucleated cells $\times 10^3/\mu\text{l}$	3.6 \pm 0.6	7.4 \pm 1.9	15.4 \pm 4.3
PMN $\times 10^3/\mu\text{l}$	2.7 \pm 0.5	1.6 \pm 0.4	4.2 \pm 2.4
M/M $\times 10^3/\mu\text{l}$	0.8 \pm 0.1	5.5 \pm 1.5	10.3 \pm 4.0
Lymphs $\times 10^3/\mu\text{l}$	0.1 \pm 0.0	0.3 \pm 0.1	1.0 \pm 0.4
MCN, 10 mg/kg	n = 7	n = 7	n = 7
Nucleated cells $\times 10^3/\mu\text{l}$	3.2 \pm 0.7	3.0 \pm 0.7	3.9 \pm 0.5
PMN $\times 10^3/\mu\text{l}$	1.9 \pm 0.4	0.5 \pm 0.1	1.0 \pm 0.2
M/M $\times 10^3/\mu\text{l}$	1.2 \pm 0.3	2.2 \pm 0.6	2.8 \pm 0.4
Lymphs $\times 10^3/\mu\text{l}$	0.1 \pm 0.1	0.2 \pm 0.1	0.2 \pm 0.1
MCN, 5 mg/kg	n = 10	n = 8	n = 8
Nucleated cells $\times 10^3/\mu\text{l}$	3.0 \pm 0.5	3.6 \pm 1.0	2.8 \pm 0.8
PMN $\times 10^3/\mu\text{l}$	1.9 \pm 0.3	0.6 \pm 0.3	0.4 \pm 0.1
M/M $\times 10^3/\mu\text{l}$	1.0 \pm 0.2	2.8 \pm 0.7	2.2 \pm 0.7
Lymphs $\times 10^3/\mu\text{l}$	0.1 \pm 0.0	0.2 \pm 0.1	0.3 \pm 0.1

*Data are expressed as mean \pm SEM. PMN = polymorphonuclear cells; M/M = macrophages and monocytes; lymphs = lymphocytes.

†Difference between TCN and 5 and 10 mg/kg MCN ($p < 0.05$).

‡Difference between TCN and 5 and 10 mg/kg MCN ($p < 0.01$).

served mesothelium. Inflammatory cell infiltration was evident both above and below the fibroelastic membrane and consisted mainly of macrophages and interspersed neutrophils, lymphocytes, and plasma cells.

All groups had evidence of mesothelial cell damage (Fig 1, A through F). Normal rabbit mesothelium, consisting of squamous-like cells with ovoid nuclei and a thin layer of cytoplasm, was replaced with cuboidal-shaped cells and large nucleoli. Separation between cells was evident, replacing the cellular approximation characteristic of the mesothelial layer. Focal desquamation of the mesothelial layer occurred in association with fibrin deposition and was often interspersed between areas of reactive mesothelium.

The subpleural parenchyma beneath areas of mesothelial damage demonstrated hypertrophy of alveolar type 2 cells. Interstitial thickening and alveoli filled with fibrin and edema fluid were also present.

The range of morphologic change was evident in all MCN groups as well as the TCN treated rabbits.

Table 2—Pleural Fluid Volume, Adhesions, Pleural Plaque Area, and Pleural Width Following Intrapleural TCN and MCN*

	TCN, 20 mg/kg (n = 11)	MCN, 20 mg/kg (n = 12)	MCN, 10 mg/kg (n = 8)	MCN, 5 mg/kg (n = 10)
Pleural fluid, ml	33.5 \pm 4.4	39.5 \pm 3.5	23.9 \pm 4.4	26.2 \pm 4.2
No. of adhesions	51 \pm 12	23 \pm 5	29 \pm 8	21 \pm 6
% Parietal plaque area	16.6 \pm 3.4	17.5 \pm 3.4	26.3 \pm 5.3	11.5 \pm 2.7
% Visceral plaque area	30.7 \pm 6.0†	20.5 \pm 4.1	23.1 \pm 3.5	7.6 \pm 1.4
Mean visceral pleural width, μm	80 \pm 19	44 \pm 4	44 \pm 4	30 \pm 4
Mean parietal pleural width, μm	140 \pm 17	86 \pm 13	103 \pm 28	65 \pm 13

*Data expressed as mean \pm SEM.

†Difference between TCN and 5 mg/kg MCN ($p \leq 0.05$).

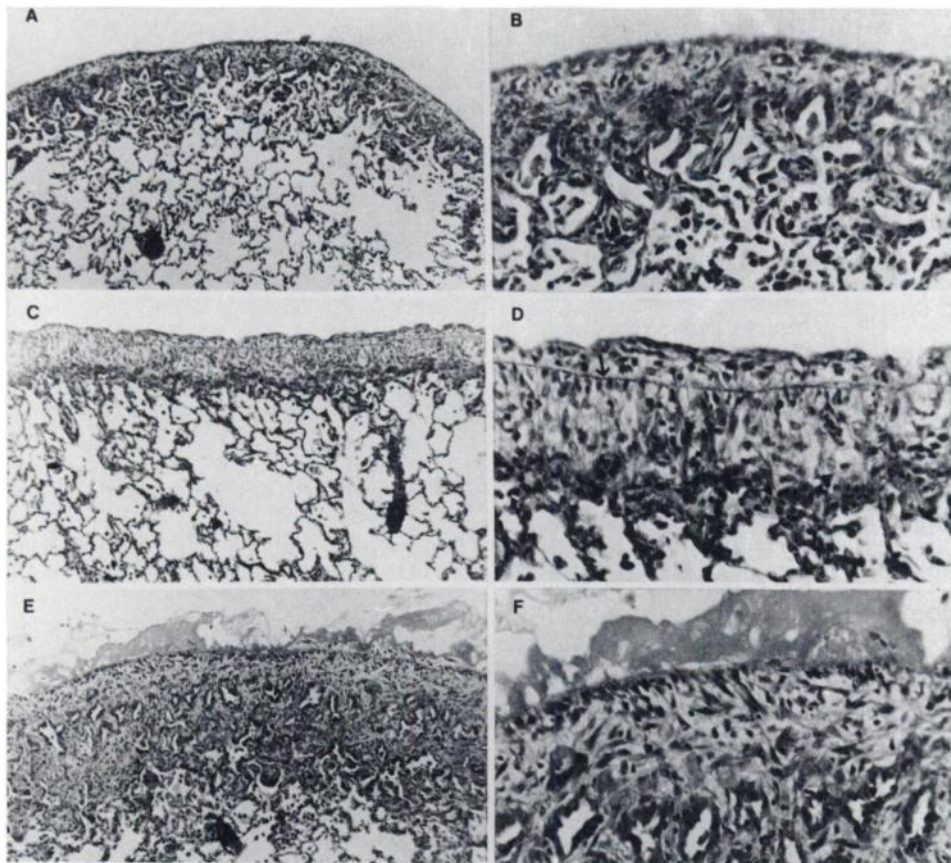


FIGURE 1. Histologic changes seen on visceral pleura following instillation of minocycline. A, C, and E are shown at $\times 180$ with B, D, and F magnified to $\times 500$. A and B: The normal mesothelium, consisting of flattened cells with ovoid nuclei, has been replaced with cellular hypertrophy, indistinct cellular borders, and nuclear enlargement (5 mg/kg MCN). C and D: Fibroblast proliferation above the elastic membrane (\rightarrow) in a 10 mg/kg MCN rabbit. E and F: Desquamation of mesothelium with loose abundant connective tissue formation with 20 mg/kg MCN. The range of morphologic change was evident in all MCN groups with higher dose MCN tending to lead to more mesothelial desquamation and connective tissue formation.

Although difficult to quantitate, intrapleural TCN and 10 and 20 mg/kg MCN tended to produce greater mesothelial desquamation and fibrin deposition compared to 5 mg/kg MCN. The 5 mg/kg dose of MCN produced less inflammatory cell recruitment and minimal subpleural parenchymal damage.

DISCUSSION

The present study was a preliminary investigation of the inflammatory response of the standard intravenous formulation of TCN and several doses of MCN on the rabbit pleura. Although the effectiveness of various chemical agents in producing pleurodesis is best assessed in human clinical trials, previous work has shown a correlation between dose-response pleural injury in the rabbit and success in clinical trials.^{3,6,16}

Predicting that MCN would have a comparable fibrotic effect is problematic. Structurally, minocycline differs from TCN by a substitution of a dimethylamino group. While this modification does little to change the antibacterial mechanism of action, physiochemical properties are altered considerably.¹⁷ MCN is highly lipophilic which may facilitate tissue penetration. Protein binding is higher with MCN compared to

TCN.^{18,19} The effect of these differences on pleural injury remains unknown.

The cellular events that occur following intrapleural instillation of TCN and MCN are similar. Both agents induce an initial neutrophil influx, followed by an increase in pleural macrophages and lymphocytes over the subsequent 48 h. A 20 mg/kg dose of MCN and TCN tends to produce a greater initial neutrophil response and subsequent macrophage and lymphocyte count compared to 5 and 10 mg/kg of MCN. The inflammatory cell response is probably an important factor in subsequent pleural fibrosis. The likely secretory products of pleural macrophages, including fibronectin, tumor necrosis factor, transforming growth factor- β , and macrophage derived growth factor may all play roles in fibroblast growth and collagen production.²⁰ Lymphokine-mediated fibroblast proliferation could also be important.²¹

We have demonstrated that equal doses of TCN and MCN produced similar volumes of pleural fluid but a trend for increased adhesions with TCN. Intuitively, the number of adhesions would appear to be critical in comparing pleurodesis agents; however, pleural fibrin formation could be equally as important. Fibrin

appears to initiate fibroblast influx and collagen formation when exposed to extracellular matrix.^{22,24} A decrease in adhesions after intrapleural streptokinase in an empyema model supports the importance of fibrin in pleural fibrosis.²⁵

Injury to the mesothelial cell or mesothelial cell basement membrane likely plays an important role in fibrin formation and fusion with the adjacent pleural surface.²⁶⁻²⁸ Mesothelial cells express procoagulant and fibrinolytic activity.²⁹ They undergo chemotaxis and proliferate in response to thrombin³⁰ and produce fibronectin, a fibroblast chemotaxin, which may play a role in pleural fibrosis.³¹ Mesothelial cell injury was present in all specimens, and fibrin formation was greatest in areas of mesothelial cell desquamation.

Minocycline, 20 mg/kg, instilled intrapleurally appears equivalent to TCN, 20 mg/kg, in the rabbit model as measured by cellular characteristics, adhesion formation, and plaque formation. Low dose MCN (5 mg/kg) produced a less inflammatory pleural effusion and little macroscopic and microscopic evidence of pleural reaction. Although the side effect profile, especially vestibular dysfunction, has limited the intravenous MCN dose in humans from exceeding 400 mg/day,^{32,35} higher doses may be tolerated for short intervals in the pleural space. Although some reports of the successful use of MCN as a sclerosing agent in humans have used less than 5 mg/kg body weight,^{12,36} higher doses would be likely to increase the success of pleurodesis. Further study is needed to assess the safety and efficacy of higher dose MCN for pleurodesis in humans.

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