Eur J Cancer Clin Oncol, Vol. 21, No. 7, pp. 875–880, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Pulmonary Toxicity in Mice after High-dose Methotrexate Administration with and without Leucovorin Rescue*

CARLO PESCE,†‡ CARLO MANSI,§ GIUSEPPE BOGLIOLO,|| FRANCA TOBIA† and IVO PANNACCIULLI

†*I Cattedra di Anatomia Patologica,* §*Cattedra di Gastroenterologia and* ||*III Cattedra di Patologica Medica, Università di Genova, Genova, Italy*

Abstract—*Interstitial lung lesions were induced in mice by high-dose methotrexate with high frequency. They appeared early after treatment; their onset, evolution and recovery parallelled those of lesions to the hemopoietic tissues and the intestine. The pathogenesis of methotrexate lung toxicity in mice is discussed. Leucovorin rescue was ineffective in preventing the lung lesions induced by high-dose methotrexate.*

INTRODUCTION

IN MAN diffuse lung disease after methotrexate (MTX) therapy has been occasionally reported in the last 15 years: more than 40 well-documented cases were reviewed in 1981 [1]. In many cases they followed long-term MTX administration. In opportune clinical settings MTX is now used in a single high dose (100 mg/kg body wt or more) followed by leucovorin (LV, N^5-formyltetrahydrofolate) rescue, which is known to prevent some of the side-effects of MTX on the hemopoietic tissues and on the intestinal mucosa [2]. There are some data suggesting that LV is not effective in preventing pulmonary toxicity in man [1, 3].

In this experimental model mimicking the treatment schedule now employed in cancer chemotherapy, either high-dose methotrexate (HDMTX) or the HDMTX-LV association was administered to mice that were killed at various time points after treatment to investigate MTX toxicity to the lung and possible LV rescue.

Accepted 24 January 1985.
*This investigation was in part supported by Contract 84.00709.44, Progetto Finalizzato "Controllo Crescita Neoplastica", Consiglio Nazionale delle Ricerche, Roma, Italy.
‡To whom requests for reprints should be addressed at: Istituto di Anatomia Patologica, via De Toni 14, 16132 Genova, Italy.

MATERIALS AND METHODS

Mice

C57BL + C3HF1F1 mice of either sex, aged 8–12 weeks, were randomly bred and maintained in a conventional environment with pellet food and water *ad libitum*.

Treatment

The mice were divided at random into three groups, A, B and C.

Group A mice received a single i.p. dose of 500 mg/kg body wt of MTX (Lederle Laboratories Division, American Cyanamid Co., Wayne, N.J.) dissolved in 0.5 ml of saline. The mean dose of MTX was 11.67 mg per mouse, corresponding to about 1.6 g/m² in man by the conversion factor of Freireich *et al.* [4].

Group B mice received HDMTX in the same way and at the same dose as group A. They were also treated with LV (American Cyanamid Co.) dissolved in saline during the 24 hr following the MTX single high dose. The initial i.v. dose of 14.5 mg/kg body wt at 2 hr was followed by an i.m. dosage of 2.5 mg/kg body wt at 6, 12, 18 and 24 hr.

Group C (control) mice were injected with 0.5 ml of saline i.p.

A preliminary trial had shown that the survival rate was 4/12 for group A mice and 12/12 for groups B and C. Accordingly, the number of mice in group A was four-fold that of mice in groups B

and C to secure at least five mice for termination per time point.

On days 1, 2, 3, 5, 8, 11, 17 and 21, at least five mice of each group were killed by cervical dislocation after slight ether anesthesia.

Histology

The lungs of each mouse were fixed immediately after termination in neutral buffered 10% formol and embedded in paraffin. Of each block, multiple sections were cut at 4 μm and stained with hematoxylin-eosin, Gram's, Grocott's (silver methenamine impregnation) and Lendrum's (phloxine-tartrazine) methods, as reported by Thompson [5], and with toluidine blue (pH 0.5) according to Strobel *et al.* [6] for mast cells. Selected sections were also stained by van Gieson's and Weigert's (fibrin) methods.

RESULTS

Group A mice

In 13/45 mice of group A, the lungs showed patchy areas of damage at low magnification. At higher magnification there was thickening of the alveolar wall and an increase in the lymphocytic and macrophagic populations of the interstitium that bulged into the alveolar spaces, causing partial or total atelectasis (Fig. 1). Few mast cells were present. Gram's and Grocott's methods failed to show pathogens in any of our mice. Lendrum's method did not show inclusion bodies, and giant cells suggesting viral infection were not seen in hematoxylin-eosin preparations.

Group B mice

In 14/43 mice of group B there were patchy lung lesions of the same type as those reported in group A mice.

In two specimens of mice killed 5 and 8 days after treatment the lymphocytic infiltrates were more prominent, and constituted perivascular and occasional peribronchial cuffs (Fig. 2). Some vessels showed endothelial proliferation and exudation of neutrophils.

Table 1 gives the frequency of lung lesions per time point in mice of groups A and B.

Group C mice

Forty mice of group C were killed, five per time point. Their lungs showed neither mononuclear infiltrates nor patchy atelectasis.

DISCUSSION

In our experience, interstitial lung lesions were found with rather high incidence, i.e. in about one out of three mice administered either HDMTX or HDMTX-LV. They most often corresponded to mild inflammatory infiltrates of lymphocytes, monocytes and histiocytes with a patchy distribution. Such lesions showed early onset, and reached a peak on days 2-5. They fully recovered within 17 days after treatment, and on day 21 no evidence of damage was found. Their evolution is very similar to the short-term MTX toxicity of hemopoietic and intestinal tissues in man and mice [2, 7].

No differences were seen in the histological pattern of interstitial lung lesions between the mice administered HDMTX only (group A) and those administered HDMTX-LV (group B), showing that LV does not prevent HDMTX toxicity to the lung. On the contrary, LV substantially reduced myelodepression and intestinal damage caused by HDMTX in mice of the same strain treated with the same HDMTX-LV schedule [8, 9].

Results of our experiment on HDMTX toxicity to the lung cannot be directly related to MTX pulmonary toxicity as it has been reported in man. The latter has been observed in diseased subjects, usually after variously lasting times of administration, in most cases with rather low doses of MTX. In several reports, the damage was evaluated only clinically. In our experiment, normal mice were used, MTX was given in a single very high dose, and results were assessed from a histological viewpoint and related to time.

Rodents provide a defective model of human lung disease, since the epithelial cell types and the cell turnover of their pneumocytes differ from those of man [14]. These differences may affect toxicological studies of cycle-dependent drugs such as MTX. Actually, lung lesions in mice showed most of the histological features that are

Table 1. Mice killed and mice affected by interstitial lesions after administration of either HDMTX or the HDMTX-LV association

Days after administration		1	2	3	5	8	11	17	21	Total
HDMTX	No. killed	7	5	6	6	5	5	6	5	45
	No. affected	2	3	2	3	2	1	0	0	13
HDMTX-LV	No. killed	5	5	7	5	5	5	5	6	43
	No. affected	1	2	2	3	2	2	2	0	14

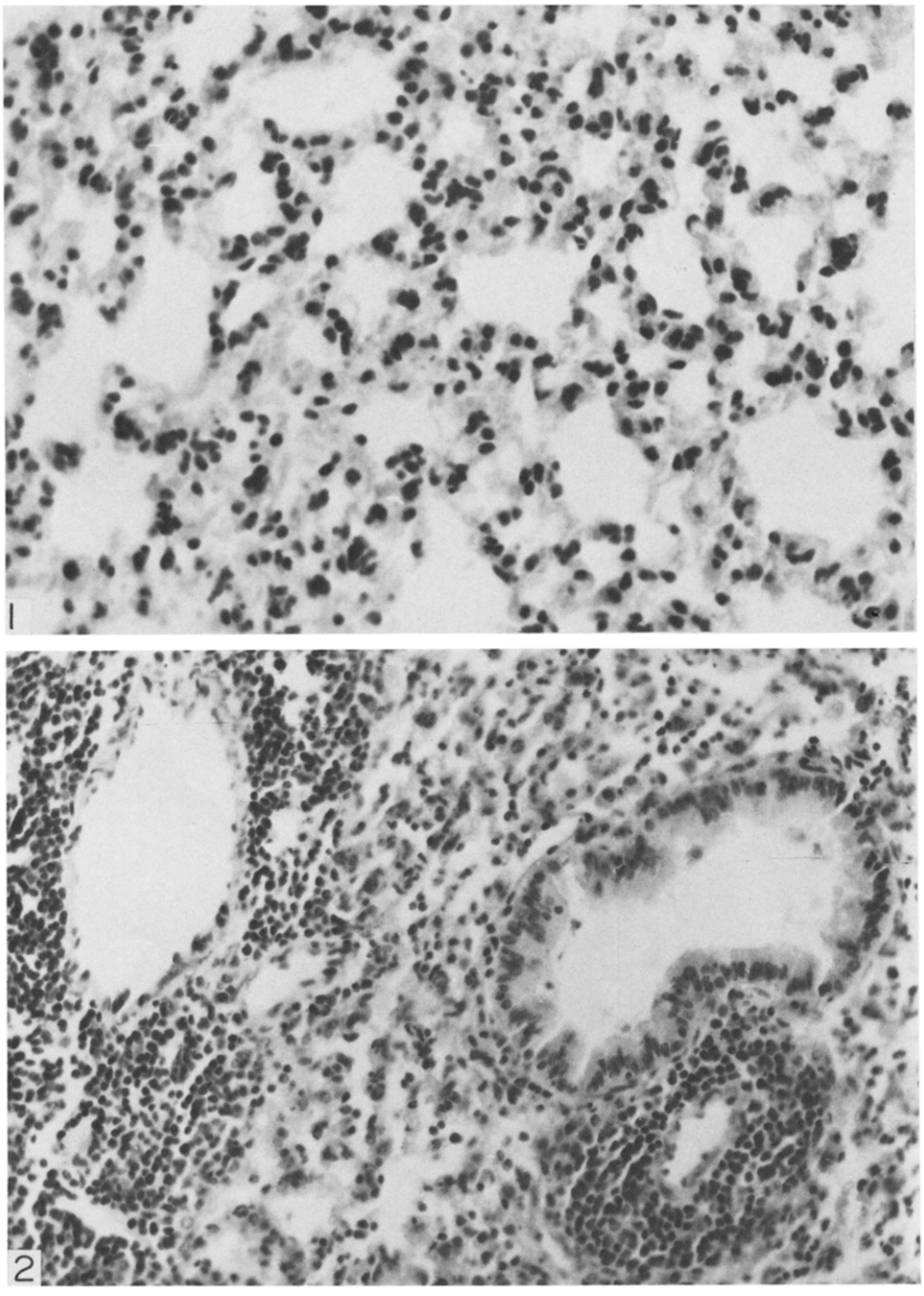

Fig. 1. *Thick alveolar walls including lymphocytes and macrophages bulge into the alveolar spaces.* (*Hematoxylin-eosin,* × 250.)

Fig. 2. *Perivascular cuffs of lymphocytes.* (*Hematoxylin-eosin,* × 100.)

present in man [11-13]. However, epithelioid and giant cells [11, 12], and fibrosis [10, 13] were absent from our specimens. Neither full-blown granulomas nor fibrosis could be otherwise expected in short-term toxicity. Lung vasculitis was present in only two instances in our experiment. It has been occasionally reported also in man [11].

LV rescue has not been shown to prevent lung disease in man [1, 3], a finding confirmed in our work, where no differences were found in the histological pattern of interstitial lesions between group A and group B mice.

The pathogenesis of MTX-induced lung disease in mice and humans is still obscure. A first consideration is that a direct cause-effect relationship is controversial. Other causes of damage to the lung, particularly infectious agents, must be ruled out [7, 11, 13]. Significantly, most patients receiving MTX therapy are immunodepressed, and MTX itself is an immunosuppressive drug. On the other hand, laboratory rodents are frequently affected by mycoplasmal and viral infections [14], which can occasionally interfere with the evaluation of the histological appearance of their lungs. Our specimens were remarkably free of giant cells, and no inclusion bodies were seen with Lendrum's method. Gram's method and silver impregnation techniques have been suggested to identify histologically infections from bacteria, fungi and *Pneumocystis carinii* in lung lesions possibly induced by MTX [7]. Both Gram's and Grocott's methods failed to show pathogens in the lung tissue. Therefore no infectious agents appeared to be involved in our experiment.

Two direct mechanisms of MTX toxicity to the human lung, namely hypersensitivity [11] and direct metabolic damage [7, 15], have been suggested, but neither has been proved. Evidence for a hypersensitivity reaction includes the latency phase, the histological demonstration of eosinophils in the inflammatory infiltrates and of granuloma formation [11], and the therapeutic response to steroids [16]. On the other hand, the latency phase is erratic [10, 17, 18], lung disease does not recur if MTX administration is resumed, which would be the rule in hypersensitivity [7], and an immnunosuppressive drug such as MTX is an unlikely agent of hypersensitivity reactions.

In our experience in mice the latency phase was generally very short. We could recognize neither a substantial increase in mast cell population nor eosinophilic infiltrates in our specimens. Most important of all, the high percentage of mice affected cannot be easily allied to an immunological mechanism. The high frequency of lung lesions found in mice that had received a very high, although single, dose of MTX may definitely point to a direct toxic action of MTX. The demonstration of a relevant concentration of MTX in the lung tissue of experimental animals could support this possibility [19]. In man, however, no definite dose-effect relationship has been reported, as we mentioned previously. Furthermore, the epithelial cells lining the bronchi, as well as type II pneumocytes, have replication phases that are remarkably longer than those of hemopoietic and intestinal cells [20]. Since MTX action is strictly related to the kinetics of its target cell populations, the low proliferating fraction makes the pulmonary tissue an unlikely target of MTX direct toxicity.

REFERENCES

1. Batist G, Andrews JL Jr. Pulmonary toxicity of antineoplastic drugs. *JAMA* 1981, **246**, 1149-1153.
2. Chabner BA. Methotrexate. In: Chabner BA, ed. *Pharmacologic Principles of Cancer Treatment*. Philadelphia, PA, WB Saunders, 1982, 229-255.
3. Ginsberg SJ, Comis RL. The pulmonary toxicity of antineoplastic agents. *Semin Oncol* 1982, **9**, 34-51.
4. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. *Cancer Treat Rep* 1977, **61**, 549-558.
5. Thompson SW. *Selected Histochemical and Histopathological Methods*. Springfield, IL, C.C. Thomas, 1966.
6. Strobel S, Miller HRP, Ferguson A. Human intestinal mucosal mast cells: evaluation of fixation and staining techniques. *J Clin Pathol* 1981, **34**, 851-858.
7. Sostman HD, Matthay RA, Putman CE, Walker Smith GJ. Methotrexate-induced pneumonitis. *Medicine* 1976, **55**, 371-388.
8. Pannacciulli I, Massa G, Bogliolo G, Ghio R, Sobrero A. Effects of high-dose methotrexate and leucovorin on murine hemopoietic stem cells. *Cancer Res* 1982, **42**, 530-534.

9. Mansi C, Savarino V, Bogliolo G *et al.* Effects of leucovorin rescue on methotrexate-damaged enteric mucosa. In Proceedings of the XII International Congress of Gastroenterology, Lisbon, 1984.
10. Rohatgi PK, Halvarson L. Unilateral methotrexate-induced pneumonitis. *J Natl Med Assoc* 1980, **72,** 896-899.
11. Clarysse AM, Cathey WJ, Cartwright GE, Winthrobe MM. Pulmonary disease complicating intermittent therapy with methotrexate. *JAMA* 1969, **209,** 1861-1864.
12. Gutin PH, Green MR, Bleyer WA, Bauer VL, Wiernik PH, Walker MD. Methotrexate pneumonitis induced by intrathecal methotrexate therapy. *Cancer* 1976, **38,** 1529-1534.
13. Nesbit M, Krivit W, Heyn R, Sharp H. Acute and chronic effects of methotrexate on hepatic, pulmonary, and skeletal systems. *Cancer* 1976, **37,** 1048-1054.
14. Masse R, Fritsch P, Chrétien J. Renouvellement des cellules des bronches et du poumon. Analyse des données récentes. *Rev Fr Mal Resp* 1981, **9,** 85-112.
15. Filip DJ, Logue GL, Harle TS, Farrar WH. Pulmonary and hepatic complications of methotrexate therapy of psoriasis. *JAMA* 1971, **216,** 881-882.
16. Schwartz IR, Kajani MK. Methotrexate therapy and pulmonary disease. *JAMA* 1969, **210,** 1924.
17 Bernstein ML, Sobel DB, Wimmer RS. Noncardiogenic pulmonary edema following injection of methotrexate into the cerebrospinal fluid. *Cancer* 1982, **50,** 866-868.
18. Lascari AD, Strano AJ, Johnson WW, Collins JGP. Methotrexate-induced sudden fatal pulmonary reaction. *Cancer* 1977, **40,** 1393-1397.
19. Anderson LA, Collins GT, Ojima Y. A study of the distribution of methotrexate in human tissues and tumours. *Cancer Res* 1970, **30,** 1344-1348.
20. Bolduc P, Reid L. Mitotic index of the bronchial and alveolar lining of the normal rat lung. *Am Rev Resp Dis* 1976, **114,** 1121-1128.