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Metronidazole: Tumorigenicity Studies in Mice, Rats and Hamsters

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Abstract

Increased incidences of lung tumors and lymphoma have been reported in Swiss mice exposed to metronidazole, but female Sprague-Dawley rats exposed to the drug did not develop an excess of any kind of tumor. Negative results have also been obtained in two hitherto unpublished carcinogenicity tests on hamsters. It is suggested that the effect in mice might be a nonspecific consequence of the antimicrobial effect of metronidazole on the gut flora. Nonetheless, the lifetime dose (per kg of body weight) of metronidazole associated with increased tumor incidence in mice was over 420 times that required for the successful treatment of trichomoniasis in humans, and lifetime doses of 400 to 500 times the trichomoniasis treatment dose had no effect on tumor incidence in hamsters.

Mouse

In a study reported in 1972 by Rustia and Shubik,[1] mice were fed on a pelleted diet containing concentrations of metronidazole ranging from 0.5% down to 0.06% from the age of six to eight weeks until they died. The diet was provided ad libitum. Treatment had no adverse effect on either survival or body weight. The findings in respect of tumor incidence are summarized in Table I. The interpretation of these data is complicated by the fact that mice in the various groups were not strictly comparable. Thus, the high-dose and control groups received oxytetracycline in the drinking water during two periods each of two weeks' duration, whereas mice in the other groups did not. Also, mice of the three lower-dose groups were, at some stage, transferred from Chicago to Omaha, whereas this was not so for the high-dose and control groups. Despite these defects in the data, it is reasonable to conclude that exposure to metronidazole was probably responsible for the increased incidence of lung tumors in male and female mice and the incidence of malignant lymphoma in female mice. The authors themselves concluded that treatment with metronidazole was not associated with a significantly increased incidence of tumors of other kinds.

Rat

In 1973, Cohen et al.[2] reported a rat carcinogenicity study on 18 different nitroaromatic compounds, including metronidazole. The study was confined to females of the Sprague-Dawley strain. Rats received metronidazole mixed with

Table I. Incidence of tumors in metronidazole-treated and control Swiss mice (from Rustia and Shubik[1]).

% Metronidazole in diet over lifespan	Number of mice examined postmortem	% Lung tumor	% Malignant lymphoma	% Other tumors
Males				
0.5	35	77	26	14
0.3	18	67	22	33
0.15	19	58	16	21
0.06	9	33	22	11
None	70	18	21	9
Females				
0.5	36	44	50	22
0.3	20	70	50	20
0.15	20	50	25	35
0.06	10	40	30	20
None	70	20	22	16

the diet at a concentration of 0.135% for up to 66 weeks. Table II summarizes the main findings in respect of tumor development. The authors concluded that the result of their study was negative.

Hamster

Dr. Rustia of the Eppley Institute (pers. commun.) fed hamsters on diets containing 0.3% or 0.15% metronidazole during alternate weeks from the age of seven weeks until death. Treatment was associated with significantly prolonged survival in both groups and both sexes compared with untreated control animals (Table III). Tumor incidence was not affected by metronidazole (Table IV).

In a study recently completed by Lowe and Ingham,[4] the effects of metronidazole administered in the diet were studied in hamsters of the Lakeview Strain, as supplied by Charles River Breeding Laboratories, Wilmington, USA. Metronidazole was given mixed in with the diet at 0, 30 or 80 mg/kg/day from the age of eight weeks until death. There were dose-related adverse effects on

Table II. Tumor incidence in female metronidazole-treated and control Sprague-Dawley rats (from Cohen et al.[2])

% Metronidazole in diet (0–66 weeks)	Max. total dose	% Mammary tumors	% Malignant mammary tumors	% Malignant tumors of other sites
0.135	10.3 gm	42	8	3
0	—	25	8	1

Table III. Effect of metronidazole on survival of hamsters (Rustia, pers. commun., 1975).

% Metronidazole in diet (alternate weeks)	Sex	0	60	80	100	120
0	♂	49	33	16	12	0
	♀	49	23	16	0	
0.15	♂	37	32	31	21	6
	♀	36	28	17	6	3
0.3	♂	35	22	21	18	7
	♀	36	22	4	11	5

body weight and food intake in both sexes. However, those effects were associated with dose-related beneficial effects on survival (Table V). Exposure to metronidazole had no effect on tumor incidence in either sex (Table VI).

Discussion

The results of other carcinogenicity tests in mice and rats are described by Dr. Rustia in another paper. These do not change the overall picture. Taken at their face value, the results of the four studies referred to above were positive in the mouse and negative in the rat and in the hamster (two studies). The negative result reported by Cohen et al.[2] was obtained in a study limited to animals of only one sex exposed to metronidazole at only one dose level. This limits the confidence to be placed on it, but no such limitation applies to the interpretation of the two hamster studies.

Table IV. Tumor-incidence in hamsters in a study carried out at the Eppley Institute (Rustia, pers. commun., 1975).

% Metronidazole in diet (alternate weeks)	Proportion with tumors	Total tumors	Total malignant tumors
Males			
0	8/46	9	3
0.15	7/32	8	2
0.3	5/26	5	2
Females			
0	7/47	10	5
0.15	3/34	3	1
0.3	5/27	6	3

Table V. Effect of metronidazole on survival of hamsters in study carried out by Lowe and Ingham.[4]

mg/kg/day Metronidazole	Sex	Survivors (weeks) 0	60	80	93
0	♂	50	36	20	13
	♀	50	34	10	—
30	♂	36	30	17	13
	♀	36	27	13	—
80	♂	36	33	24	18
	♀	36	29	13	—

In the case of the mouse study of Rustia and Shubik,[1] the findings present several worrying or puzzling features. Firstly, the kinds of tumor which occurred in significantly higher incidence in metronidazole-treated mice were also found relatively commonly in untreated control animals. Thus in both sexes, approximately 20% of the mice developed lung tumors and 20% developed malignant lymphoma. Clearly there are other factors — genetic or environmental — which play a major role in determining the occurrence of these tumors. In 1974, Mary Tucker and I[3] described how relatively slight dietary restriction can reduce by as much as eight-fold the incidence of lung tumors and malignant lymphoma in Swiss mice (Table VII). Metronidazole is an antimicrobial agent which is especially active against anaerobic bacteria; indeed, for this reason it has been found to have an important clinical use in preventing and treating infections due to anaerobic bacteria of gut origin. It is to be expected, therefore, that prolonged exposure to metronidazole will affect nutritional status, particularly under conditions of continuous exposure. This possibility needs to be borne in mind in the interpretation of carcinogenicity tests, especially on mice and particularly in relation to the occurrence of lung tumors and malignant lymphoma in mice.

Table VI. Tumor incidence in metronidazole-treated and control hamsters in study carried out by Lowe and Ingham.[4]

mg/kg/day Metronidazole	No. of animals	Total tumors	Total malignant tumors
Males			
0	50	13	3
30	36	3	1
80	36	10	3
Females			
0	50	9	1
30	36	3	0
80	36	4	1

Table VII. Effect of dietary intake on incidence of liver, lung and other tumors in Swiss albino male mice maintained under specified pathogen-free conditions (from Roe and Tucker[3]).

Group	No. of mice	Feeding +	Total tumors by 18 months	Liver tumors	Lung tumors	Lympho-reticular neoplasms	Other neoplasms
1	40	4 gm diet/day 1 mouse/cage	4	1	1	2	0
2	40	5 gm diet/day 1 mouse/cage	4	2	0	1	1 testis
3	40	Diet ad libitum 1 mouse/cage	32	15	2	11	2 testis 1 kidney 1 thyroid
4	40	Diet ad libitum 5 mice/cage	23	8	6	9	0

Table VIII. Comparison of doses given in carcinogenicity tests with those used clinically in the treatment of trichomoniasis.

Study	Species	Dose (mg/kg/day)		Total dose by 80 weeks (mg/kg)	Multiple of one course of treatment for trichomoniasis‡	Result of carcino-genicity test
Rustia and Shubik[1]	Mouse	(Highest)	625*	350×10^3	3,500	+
		(Lowest)	75*	42×10^3	420	?
Cohen et al.[2]	Rat	?		40×10^3	400	–
Rustia (pers. commun.)	Hamster	(High)	168†	47×10^3	470	–
Lowe and Ingham[4]	Hamster	(High)	80	45×10^3	450	–

* Assuming that a mouse eats 5 gm of diet per day and weighs 40 gm.
† Assuming that a hamster eats 7 gm of diet per day and weighs 125 gm.
‡ 10 mg/kg/day for 10 days.

Another interpretative problem in the case of the Rustia and Shubik study[1] is that treatment was associated with an increased incidence of malignant lymphoma in females but not in males. This difference begs explanation. A third problem is the lack of any clear dose-related trend for lung tumor incidence in females, although this may be because the mice in the highest-dose group were not strictly comparable with those in other groups.

Without further information, it is clearly not possible to distinguish between two hypotheses in relation to the apparent effect of metronidazole on the incidence of lung tumors and malignant lymphoma in mice:

1.) Metronidazole *induced* the additional tumors seen in the treated as compared with the control groups.
2.) Metronidazole *increased* the risk of development of lung tumors and malignant lymphoma which arise 'spontaneously' in mice because of previous exposure to background tumor-inducing agents (e.g., vertically transmitted viruses, defective genes, environmental chemicals).

This brings me to perhaps the most important single consideration. In Table VIII, I have attempted to compare the doses of metronidazole given to animals in the four studies I have been discussing with those given to patients in the clinic for the treatment of trichomoniasis. In preparing this Table, I have had to make certain assumptions and approximations (see footnotes to the Table). Nevertheless, the message I feel is abundantly clear: lifetime doses 400 to 500 times the total dose necessary for the treatment of trichomoniasis in humans, on a mg/kg basis, gave negative results in respect of carcinogenicity in hamsters, while the positive result recorded in mice by Rustia and Shubik,[1] whatever its explanation, entailed their exposure to at least 420 times the trichomoniasis treatment dose.

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