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Safety Evaluation of Metronidazole From the Viewpoint of General Toxicity And Carcinogenicity 1981 Appraisal

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ABSTRACT

A review of studies carried out since 1979 to evaluate the carcinogenicity and toxicity of the drug metronidazole is presented.

In both laboratory animals and man, the gastrointestinal tract and the nervous system are the main targets for metronidazole toxicity. Minor side effects (nausea, bad taste in the mouth, dizziness, sleepiness, headache) occur in a small number of patients taking standard doses of metronidazole (e.g., 600 mg per day for five days or a single dose of 2 g). A disulfiram effect upon alcohol consumption and skin rashes have also been observed. Tests for embryotoxicity and teratogenicity in rats, rabbits, and mice gave consistently negative results.

The results of carcinogenicity tests are inconclusive. Metronidazole causes mutations in certain strains of S. typhimurium. *Prolonged exposure of rats and mice to very high doses increases the incidence of lung tumours in mice and possibly liver tumours in the rats. Acetamide, a metronidazole metabolite, is a weak hepatocarcinogen.*

Metronidazole has no demonstratable effect on human lymphocytes or cells maintained under hypoxic conditions. No increase in the frequency of chromosomal aberrations has been observed in patients with Crohn's disease receiving long-term metronidazole treatment or in patients with vaginal trichomoniasis on a seven-day course of treatment.

Two independent epidemiological studies show no evidence of a statistically significant increased risk of cancer at any site as a consequence of exposure to metronidazole.

INTRODUCTION

I have previously reviewed this subject in 1977 and 1979.[1-3] The main purpose of the present paper is to discuss the results of research since the last of these reviews.

GENERAL TOXICITY

On this topic, there is nothing to add to my last review.[3] Reversible minor side effects occur in a minority of patients given the kinds of doses usually recommended for the treatment of trichomoniasis caused by *T. vaginalis* (e.g. 600 mg per day for five days or a single dose of 2 g). These commonly affect the gastrointestinal tract, causing nausea or bad taste in the mouth, or the nervous system,producing dizziness,sleepiness, or headache. Rashes are occasionally seen, and a disulfiram effect on taking alcohol has been reported. In response to higher and/or more prolonged exposure to the drug, such as when it has been given as a radiosensitizer, more severe gastrointestinal effects have been reported, including

vomiting and anorexia. Also, more severe effects on the central and peripheral nervous systems—transient encephalopathy, paraesthesias, peripheral neuropathy—have been described. The gastrointestinal symptoms usually clear up on withdrawal of the drug, but persistence, or only slow recovery, has occasionally been encountered in the case of neurotoxic effects. In laboratory animals, as in man, the gastrointestinal tract and nervous system are the main targets for toxicity.

Properly designed tests for embryotoxicity and teratogenicity in rats, rabbits, and mice have consistently given negative results.[4] A report of increased incidence of birth defects, stillbirths and premature births in rats, mice, and guinea pigs[5] is unacceptable because of poor experimental design and methodology, inadequate group size and lack of proper controls. Metronidazole has been widely used in the treatment of *Trichomonas* sp. infections at all stages of pregnancy without any reports of teratogenic effect. However, most clinicians these days feel it prudent to avoid if possible the use of the drug during the first trimester.

Results of Carcinogenicity Studies in Animals

Until 1979, the results of seven carcinogenicity studies in animals have been reported—three in mice, two in rats, and two in hamsters.[3] The results of the studies in hamsters were unequivocally negative, but those in rats were somewhat equivocal, particularly because both were only 80 weeks in duration. Enhanced lung tumour risk was a common feature in the three mouse studies, all of which were conducted in Swiss mice, in which such tumours occur endemically in high incidence.

Additionally, in one of the three studies,[6] a raised incidence of lymphoreticular neoplasms was reported in females. Again, the background incidence of these tumours in the untreated controls was high (more than 20%), so that it is possible that the increased incidence was a nonspecific effect rather than one attributable to tumour-induction by the drug. Elsewhere[7] I have suggested that high background incidences of neoplasms may be a laboratory artefact due to overfeeding. In all three mouse studies mentioned above, the mice were fed *ad libitum*. Slight dietary restriction has been reported to reduce dramatically the incidence of a wide variety of neoplasms in mice, including lung tumours and lymphoreticular neoplasms.[8,9] Metronidazole has pronounced effects on the gut flora, selectively killing anaerobic bacteria. In a long-term feeding study, this activity is likely to result in a change in the nutritional status of animals. Such a change might well have enhanced the incidences of lung tumours and of lymphoreticular neoplasms in the Rustia and Shubik study,[6] nonspecifically.

Since 1979, the results of a further rat study have become available.

Rat Study Report by Rustia and Shubik

In this study, groups of 30 male and 28-30 female rats of the noninbred strain, Sas:MRC(W1)BR, were exposed to 0.6%, 0.3%, or 0.06% metronidazole in the diet, with 97 untreated males and 100 untreated females serving as controls. In both sexes, treatment at all dose levels was associated with significantly better survival. In males, the 50% survival point was reached at about 88 weeks in the controls but not until 30 weeks later in any of the treated groups. In females, the 50% survival point was reached at about 106 weeks in the controls and not until between 114 and 126 weeks in the treated groups (Figures 1 and 2). Since the incidence of most forms of neoplasm increases logarithmically with age and since most of the neoplasms recorded in the study were found incidentally at necropsy (i.e., they did not contribute to the cause of death), meaning-

Figure 1

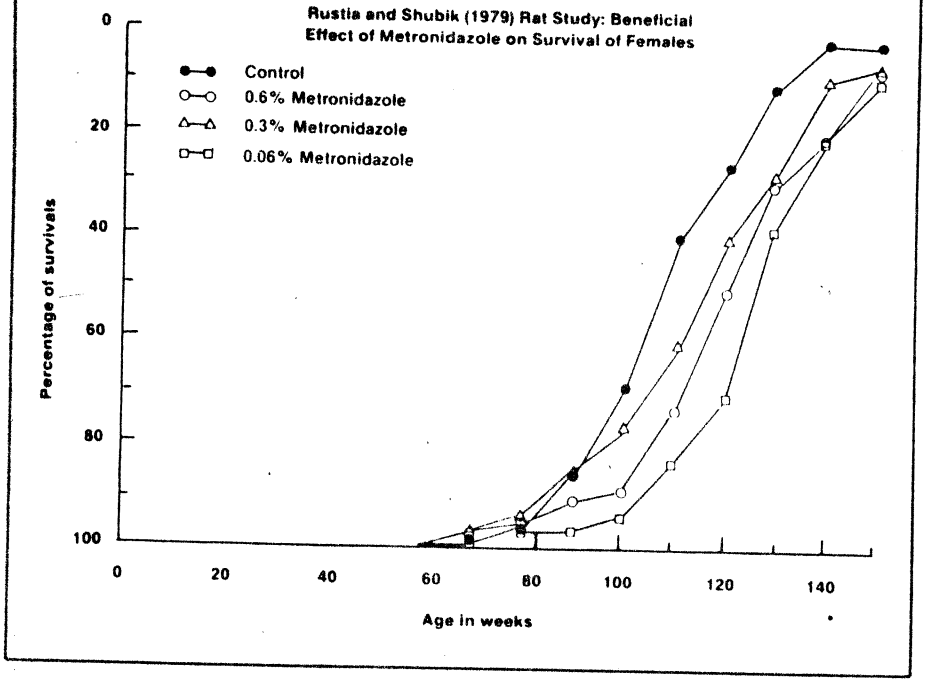
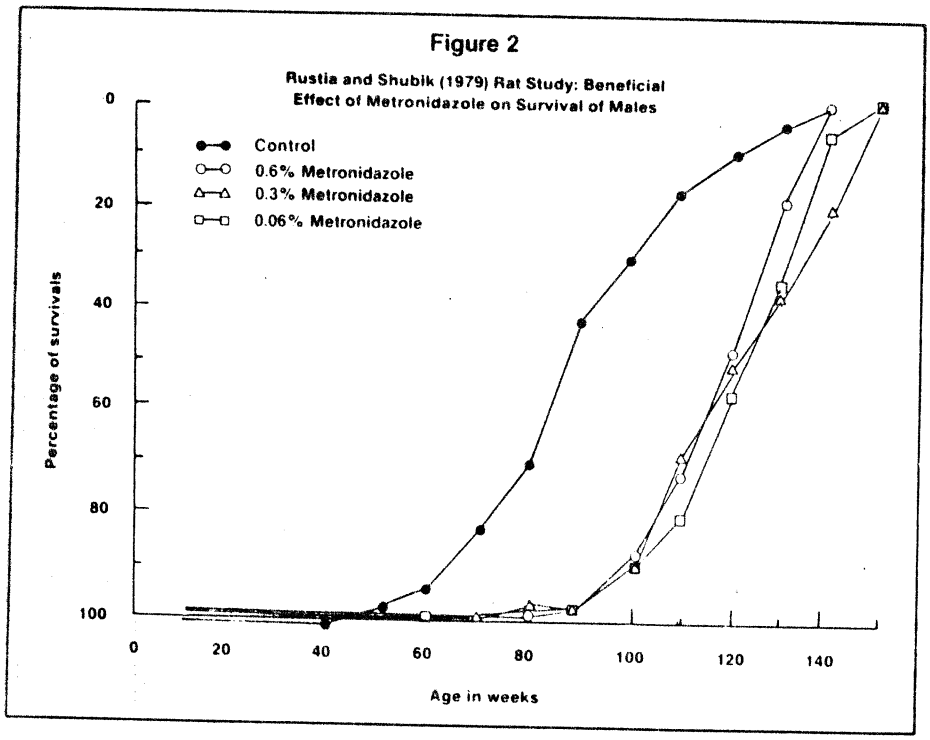


Figure 2



ful comparison of tumour incidences between groups could have been made only by means of the statistical approach recommended by IARC (1980).[10] Unfortunately, the authors report only the crude tumour data, totally ignoring the very great survival differences. Consequently, their conclusions with regard to adverse effects of metronidazole on tumour incidence* are misleading. It would be my guess that after age-standardisation, the only adverse difference in tumour incidence likely to remain as statistically significant would be the excessive incidence of liver tumours in the top-dose female group. In this connection it is noteworthy that Rustia and Shubik considered some of the liver tumours in the top-dose female group to be malignant, although none of them had metastasised to the lung or other sites.

*Mammary tumours in females increased $p<0.02$; liver cell tumours in females increased $p<0.05$; Leydig cell tumours of the testis increased in males $p<0.04$; pituitary tumours increased in high-dose males $p<0.04$ (Tables 1 and 2).

Overview of Results of Animal Carcinogenicity Studies

The suspicion arising from the Rustia and Shubik[6] rat study that an excess of liver tumours may occur in female rats exposed continuously to 0.6% metronidazole in the diet does not change the overall picture. Assuming that the rats in question on the average weighed 300 g and ate 15 g food per day, the rate at which they were continously exposed to metronidazole throughout life was about 300 mg/kg/day. This dosage is of the same order as that associated with enhanced lung tumour incidence in mice; that is to say, in terms of life-time exposure, it is equivalent to 3,000 times or more the total dose given to humans for the treatment of trichomoniasis (assumed to be 10 mg/kg/day for 10 days). Without further research it would be impossible to guess the mechanism(s) involved in any enhancement of liver tumour risk in rats. Alternatives include:

1) tumourigenic metabolites of metronidazole produced *pro rata* irrespective of exposure level;
2) tumourigenic metabolites of metronidazole produced only under conditions of excessive exposure (such as when a detoxifying mechanism is overwhelmed);
3) an indirect effect due to a change in gut flora (e.g., bacteria in the gut of high-dose metronidazole-treated animals produce enzymes which facilitate the production of carcinogenic metabolites from food constituents or from bile acids, etc.);
4) liver tumour incidence is non-specifically increased as a consequence of metronidazole's effect on nutritional status.

Acetamide as a Carcinogenic Metabolite of Metronidazole

Koch et al [11] published persuasive evidence that acetamide may be formed from metronidazole by intestinal flora. Yields of between 8% and 15% were obtained when metronidazole was incubated with rat faecal contents or with *Clostridium perfringens*, and acetamide was recovered from both the urine and faeces of rats given a 200 mg/kg dose of radio-labelled metronidazole by gavage. Its absence in the urine and faeces of similarly treated germ-free rats suggests that conversion to acetamide is dependent on the action of bacterial enzymes.

The studies of Jackson and Dessau[12] and Weisburger et al[13] indicate that acetamide is a weak hepatocarcinogen. In response to a concentration of 1.25% acetamide in the diet (the equivalent of a cumulative dose over a lifetime of the order of 250 g/kg) Jackson and Dessau saw only four liver tumours in 24 rats. To expose animals to as much acetamide as Jackson and Dessau's 1.25% dietary group one would have to

Table 1

Non-age-standardised Tumour Incidence Data From Rustia and Shubik (1979) Rat Study: Males

Metronidazole in diet (%)	No. of rats	50% survival point (weeks)	% of rats with tumours				
			Pituitary	Mammary gland	Liver	Testis	Any site
0.6	30	116	50	10	3	47	90
0.3	30	116	33	3	0	20	70
0.06	30	122	37	7	0	30	73
None	100	88	20	0	2	18	47

Table 2

Non-age-standardised Tumour Incidence Data From Rustia and Shubik (1979) Rat Study: Females

Metronidazole in diet (%)	No. of rats	50% survival point (weeks)	% of rats with tumours				
			Pituitary	Mammary gland	Liver	Uterus	Any site
0.6	30	120	57	77	23	33	100
0.3	28	114	68	53	4	29	100
0.06	27	125	80	57	0	30	90
None	80	105	63	35	0	13	82

give them about 12% metronidazole in the diet and thereby exceed the dose levels used therapeutically by almost a thousand times.

No doubt the implications of the findings that acetamide may be a metabolite of metronidazole merit further research, particularly since Rustia and Shubik's[6] study points to the liver as a possible target for increased tumour risk in rats exposed to metronidazole. However, it is very doubtful that such research will lead to the conclusion that metronidazole poses a carcinogen risk because of this metabolic pathway.

Bacterial Mutagenicity

Dr. M. Müller, elsewhere in this publication, discusses the mechanism underlying the antimicrobial activity of metronidazole. All that needs to be said here is that in anaerobic bacteria, the antimicrobial activity of metronidazole seems to be closely dependent on the reduction of its nitro-group with the formation of short-lived cytotoxic intermediates capable of reacting with DNA and that where mutations occur in certain strains of *S. typhimurium* on exposure to metronidazole, nitroreduction is probably implicated.[14]

Cytogenicity Tests

The micronucleus test in mice is a higly sensitive test system for the demonstration of cytogenic damage.

Hartley-Asp[15] found no increase in micronuclei frequency compared with controls in bone marrow preparations from mice given two doses, 24 hours apart, of up to 4,000 mg/kg metronidazole.

Under hypoxic conditions, metronidazole can be highly cytotoxic for cultured mammalian cells[16] and this has led to trials of the drug and other nitroimidazoles in the treatment of cancer, either alone or in conjunction with radiotherapy. Korbelik and Horvat[17] reported no induction of chromosomal aberrations in V79-379A Chinese hamster cell cultures after incubation with metronidazole under aerobic condi-

tions. However, they did see an increase in number of aberrations compared with control cultures after incubation with metronidazole under hypoxic conditions, and this suggested that hypoxic mammalian cells might possess latent nitroreductase activity. On the other hand, Dunlop et al[18] saw no increase in sister chromatid exchange when they exposed Chinese hamster ovary cells to up to 1,000 μM metronidazole under anoxic conditions.

Under aerobic conditions of culture, several studies have indicated that human lymphocytes do not convert metronidazole to a cytotoxic metabolite, nor do they exhibit chromosomal aberrations or show increased sister chromatid exchange on incubation with metronidazole.

Thus Hartley-Asp[15] reported experiments designed to test the effects of exposing peripheral lymphocytes obtained from healthy human donors to metronidazole *in vitro*. She observed no increase in the frequency of abnormal cells or chromosomal aberrations after the exposure of the cells to 500, 1,000, or 10,000 μg/ml metronidazole in the culture medium for 72 hours.

Earlier, Lambert et al[19] reported negative results in cultured human lymphocytes exposed to either metronidazole itself or to either of its major human urinary excretion metabolites [2-methyl-5-nitroimidazole-1-yl acetic acid and 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole] at concentrations of up to 1,000 μg/ml for as long as 75 hours. In these studies there was no increase in the frequency of chromosomal aberrations and no increase in sister chromatid exchanges. Finally, Prosser and Hesketh[20] saw no increase in the number of sister chromatid exchanges in human lymphocytes incubated for two hours in the presence of 0.5 or 8 μg metronidazole with or without the addition of S-9 microsome mix to the medium. Under the same conditions, incubation with 10^{-6} M cyclophosphamide increased sister chromatid exchanges fivefold in the presence of S-9 mix.

It appears that no one has studied the effect of metronidazole on human lymphocytes or other cells maintained under hypoxic conditions. Thus it remains uncertain whether nitro-reduction of metronidazole to potentially genotoxic metabolites could occur under exceptional circumstances in humans. If it occurs at all, it is most likely to do so in the ischaemic centres of neoplasms. In any case, an overview of the evidence suggests that nitroreduction does not occur in the presence of oxygen, which is the normal circumstance.

Absence of Chromosome Aberration in Patients Treated With Metronidazole

In a preliminary communication, Mitelman et al [21] reported a suggestively higher frequency of chromosomal abnormalities in the circulating lymphocytes of Crohn's disease patients receiving one-term metronidazole therapy than in Crohn's disease patients not receiving the drug. However, the results had to be regarded as inconclusive because no pretreatment values for frequency of chromosome breaks were available. Subsequently, the same group of workers[22] reported the results of a small double-blind crossover study. Twenty-two Crohn's disease patients were randomised into two groups, one for treatment with metronidazole (0.8 g per day) and one for treatment with sulphasalazine (3 g per day). After four months, treatments were switched. The frequency of chromosomal aberrations in peripheral blood lymphocytes was assessed before any treatment started and at one and four months after the start of each treatment. Neither form of treatment was found to be associated with any increase in the incidence of chromosomal aberrations.

In another study, Hartley-Asp[23] found no increase in chromosomal aberrations in

the peripheral lymphocytes of 12 women while they were receiving (day 7) or three weeks after they had completed, a seven-day course of metronidazole (600 mg/day) for vaginal trichomoniasis.

These negative findings are consistent with those obtained in animals and in cells under *in vitro* conditions (*vide supra*).

Epidemiological Studies

One of the main purposes of the extensive carcinogenicity testing of metronidazole in the laboratory has been to assess whether humans exposed to the drug are at excessive risk of toxicity or carcinogenicity. It goes without saying that in this regard the results of observations actually on humans are especially important and relevant.

At the 1979 conference, Dr. Beard and her colleagues reported the results of a follow-up study on 771 women treated with metronidazole for trichomoniasis at the Mayo Clinic between 1960 and 1969. Since then, a fuller report of their study has been published.[24] Apart from the 771 women treated with metronidazole, there were available for study 237 women who had been treated for trichomoniasis with drugs other than metronidazole. Ninety-three percent of the metronidazole-treated group, but only 86% of the control group, had been successfully traced at the time of the 1979 report. Among those traced, 24 cases of cancer other than of the uterine cervix were identified and compared with an expected 21.7 cases based on the Connecticut Tumor Registry data, and an expected 18.4 cases on the basis of the Third National Cancer Survey. The excess of observed over expected cases for cancers of all sites, which did not reach statistical significance at its 5% level, is probably attributable to the fact that all of the four (compared with 0.6 expected) women who developed lung cancer were smokers. From previous publications,[25,26] an excess of cases of carcinoma *in situ* of the uterine cervix was to be expected in women with a history of trichomoniasis. Beard et al[24] observed a similar excess of such cases among the women who received no metronidazole (eight observed versus 3.9 expected) as in the women who did receive the drug (21 observed versus 12.1 expected). The authors of the study concluded that it provided no substantial evidence of an excessive risk of cancer at any site as a consequence of exposure to metronidazole.

Another report of direct relevance to this issue is that of Friedman and Ury[27], which is based on the findings in the Kaiser-Permanente Medical Care Program's screening of 53 commonly used drugs for evidence of carcinogenicity in humans. Data were available on 2,460 patients who had been given at least one prescription of metronidazole between July 1969 and August 1973. Among these, there was no evidence of statistically significant increased risk of cancer of any site or of all sites combined.

The results of these studies are reassuring as far as they go, but obviously data from studies of long duration with better information on potentially confounding variables (e.g., smoking habits) will be needed before one might state with confidence that metronidazole in therapeutic doses is completely safe in terms of cancer risk.

CONCLUSIONS

The fact that metronidazole gives positive results in certain bacterial mutagenicity tests and that prolonged exposure of rats and mice to very high doses does increase the incidence of certain tumours (i.e., lung tumours in mice and possibly liver tumours in rats) indicate a need to keep under continuous review the possibility that it may predispose to one or another form of cancer in humans.