MUTAGENICITY OF METRONIDAZOLE: ACTIVATION BY MAMMALIAN LIVER MICROSOMES

Herbert S. Rosenkranz and William T. Speck

Departments of Microbiology and Pediatrics
College of Physicians and Surgeons
Columbia University
New York, New York 10032

Received July 16,1975

SUMMARY: The mutagenicity of metronidazole, a widely used chemotherapeutic agent, for Salmonella typhimurium was confirmed. Moreover using a mutant of of S. typhimurium unable to activate metronidazole to a genetically active metabolite, it is shown that this activation can be carried out by a microsomal preparation derived from rat liver. Heretofore it had been postulated that this metabolic event was catalyzed solely by enzymes present in protozoa and anaerobic bacteria. The present findings which indicate that mammalian enzymes can activate metronidazole to a genetically active intermediate may have a direct relevance to the carcinogenicity of this agent.

Metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) is widely used for the treatment of protozoan infections. Recently, use of this drug for the therapy of infections caused by anaerobic bacteria has been advocated (1). Several recent studies (2,3) have reported the mutagenicity of metronidazole for several bacterial species and in view of the correlation between genetic effects in prokaryotes and ability to induce cancers in mammals (4,5), the widespread usage of this drug to treat human infections has been questioned (6). It is presumed that the genetic activity of metronidazole is dependent upon its ability to be reduced enzymatically to the hydroxylamino or amino derivative. It may be argued, however, that this reduction is dependent solely upon the microbial (and protozoan) metabolism (7-11) and therefore the observed mutagenic activity towards bacteria is without relevance to humans who are unable (12,13) to convert metronidazole to a mutagenic (and carcinogenic) intermediate.

In this study we demonstrate the conversion of metronidazole to a genetically active intermediate by mammalian microsomes.

MATERIALS AND METHODS

Salmonella typhimurium TA100 (14) was obtained from Dr. Bruce N. Ames, University of California, Berkeley. A derivative of this strain deficient in nitro-reductase was obtained by selection (15) of bacteria resistant to nitrofurazone (20 μg/ml, 5-nitro-2-furaldehyde semicarbazone). One isolate identified as TA100-FR1 was used for these studies. The absence (4% of Control) of nitrofurazone reductase activity was confirmed colorimetrically (15). TA100-FR1 was resistant to the growth inhibitory properties of other nitrofurans, metronidazole, picrolonic acid, 2-nitrofluorene and 2-nitronaphthalene, thus indicating that this strain was deficient in a non-specific nitro-reductase.

Rat liver microsomes (S-9 fraction) were prepared as previously described (4). Mutagenicity assays using TA100 and TA100-FR1 were carried out as described by Ames and his associates (16) by incorporating the indicator strain, the test agent and when indicated the microsomal fractions (including required co-factors) into an agar overlay. The inoculated plates were incubated at 37°C for 46 hours in the dark (17) and revertants to histidine independence were enumerated. When anaerobic conditions were required, the plates were placed into a Gas-Pak jar (BBL, Cockeysville, Md.) and incubated at 37°C for 16 hours whereupon they were incubated an additional 30 hours aerobically.

RESULTS

Salmonella typhimurium TA100, the parent strain, was mutagenized by metronidazole as evidenced by the induction of revertants to histidine-independence. This effect was dependent upon the amount of drug incorporated into the plate (Table 1, Expt. I). Contrariwise, a nitro reductase deficient

Table 1

Mutagenicity of Metronidazole for Salmonella typhimurium

Expt.	Additions	µg/Plate	Microsomes	Conditions	Revertants Per Plate	
					TA 100	TA100-FR1
I	None	0	—	Aerobic	244	161
	Metronidazole	25	—	"	310	
	"	50	—	"	358	
	"	125	—	"	534	
	"	250	—	"	1171	159
II	Metronidazole	250	—	Aerobic	1357	181
	"	250	+	"	1392	227
	"	250	—	Anaerobic	1594	824
	"	250	+	"	1563	2008
	None	0	—	Aerobic	238	155
	"	0	—	Anaerobic	266	150
	"	0	+	Aerobic	233	163
	"	0	+	Anaerobic	245	175
III	None	0	+	Anaerobic		147
	Metronidazole	50	+	"		892
	"	125	+	"		1945
	"	250	+	"		1992
	"	500	+	"		1296
IV	Ethyl methane-sulfonate	7	—	Aerobic	5000	5000
	" "	7	—	Anaerobic	5000	5000
	1,2-Epoxybutane	7	—	Aerobic		408
	Sodium Azide	25	—	"		2390
	Propylenimine	1.4	—	"		7000
	2-Bromoethanol	5.5	—	"		702
	Propane sultone	7	—	"		1450

derivative (TA100-FR1) did not respond to the mutagenic action of this agent (Table 1, Expts I and II). This lack of responsiveness was not due to an inability of the tester strain to respond to mutagens as it was readily mutagenized by a series of mutagens lacking the nitro function (Table 1, Expt IV). Incubation of TA100-FR1 with metronidazole in the presence of a microsomal preparation resulted in a partial restoration of mutagenic activity (Table 1, Expt II). Incubation of the plates anaerobically for several hours resulted in expression of mutagenic activity even in the absence of added mammalian microsomal preparation (Table 1, Expt II). It appears that this reflects the presence in these bacteria of an oxygen-labile nitro reductase (unpublished results). When the plates were supplemented with a microsomal preparation and incubated anaerobically, the yield of mutants was increased further (Table 1, Expt II). This microsome-mediated enhancement of the mutagenic effectiveness of metronidazole was concentration dependent (Table 1, Expt III). It should be stressed that these manipulations had no effect on the yield of spontaneous mutations (i.e. background) (Table 1, Expt II). It is interesting that microsomes had no effect on the mutagenic effectiveness of metronidazole for TA100; presumably the bacterial enzyme is as effective as the liver preparation in converting the drug to its activated form. When incubation was allowed to proceed anaerobically, there was a slight but significant increase in the mutant yield. This presumably reflects the activity of the oxygen-labile nitro reductase possessed by these bacteria.

DISCUSSION

The ability of a mammalian microsomal preparation to "activate" metronidazole is surprising in view of the previously held assumption that this was an ability unique to anaerobic bacteria and protozoa (7-13). The present data which show that mammalian liver enzymes are capable of catalyzing the con-

version of this drug to a mutagen deserves consideration because of the accepted view that mutagenic activity can be taken as an indication of carcinogenic potential (4, 5). In this connection it is perhaps germane that the carcinogenicity of metronidazole for rodents has been reported (18).

In view of the widespread use of metronidazole and because it is possible that the human liver is capable of activating this drug to a genetically active intermediate, it would seem that the utilization of this drug should be controlled more rigorously.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. B.N. Ames, University of California at Berkeley for providing them with a specimen of Salmonella typhimurium TA100. This study was supported by the Division of Cancer Cause and Prevention, National Cancer Institute (NO 1-CP-33395). One of the authors (H.S.R.) is a Research Career Development Awardee of the National Institute of General Medical Sciences (5 K3-GM 29,024), the other (W.T.S.) is the Vivian Allen Fellow in Clinical Medicine.

REFERENCES

1. Tally, F.P., Sutter, V.L. and Finegold, S.M., Calif. Med. 117, 22 (1972).
2. Voogd, C.E., Van der Stel, J.J. and Jacobs, J.J.J.A.A., Mutation Res. 26, 483 (1974).
3. Legator, M.S., Connor, T.H. and Stockel, M., Science 188, 1118 (1975).
4. Ames, B.N., Durston, W.E., Yamasaki, E. and Lee, F.D., Proc. Nat. Acad. Sci. U.S. 70, 2281 (1973).
5. Miller, E.C. and Miller, J.A. in A. Hollaender (ed.), Chemical Mutagens, Vol. 1, Plenum Press, Inc., New York, N.Y. pp. 83-119 (1971).
6. The Medical Letter on Drugs and Therapeutics 17, 53 (1975).
7. Edwards, D.I. and Mathison, G.E., J. Gen. Microbiol. 63, 29 (1970).
8. Ings, R.M.J., McFadzean, J.A., and Ormerod, W.E., Biochem. Pharmacol. 23, 1421 (1974).
9. Coombs, G.H., in P. de Puytorac and J. Grain (eds.), Progress in Protozoology, Proc. 4th Intern. Congr. Protozoology, p. 92 (1973).
10. Lindmark, D.G. and Müller, M., in P. de Puytorac and J. Grain (eds.), Progress in Protozoology, Proc. 4th Intern. Congr. Protozoology, p. 251 (1973).
11. Lindmark, D.G. and Müller, M., J. Protozool. 21, 436 (1974).

12. Ings, R.M.J., Law, J.L., and Parnell, E.W., Biochem. Pharmacol. 15, 515 (1966).
13. Stambaugh, J.E., Feo, L.G. and Manthei, R.W., J. Pharmacol. Exptl. Ther. 161, 373 (1968).
14. McCann, J., Spingarn, N.E., Kobori, J. and Ames, B.N., Proc. Nat. Acad. Sci. U.S. 72, 979 (1975).
15. McCalla, D.R., Reuvers, A. and Kaiser, C., J. Bacteriol. 104, 1126 (1970).
16. Ames, B.N., Lee, F.D. and Durston, W.E., Proc. Nat. Acad. Sci. U.S. 70, 782 (1973).
17. Speck, W.T. and Rosenkranz, H.S., Photochem. Photobiol. 21, 369 (1975).
18. Rustia, M. and Shubik, P., J. Nat. Cancer Inst. 48, 721 (1972).