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Mutagenicity of Azathioprine[1]

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SUMMARY

Azathioprine (6-[(1-methyl-4-nitroimidazol-5-yl)thio]purine; Imuran) is mutagenic for *Salmonella typhimurium*. Demonstration of this mutagenic effect requires a period of anaerobic incubation of the bacteria with the test agent.

INTRODUCTION

Azathioprine (6-[(1-methyl-4-nitroimidazol-5-yl)thio]purine; Imuran) is widely used as an immunodepressant; moreover, this agent is also endowed with antitumor activity (5). Although azathioprine has no demonstrable carcinogenic activity, an increased incidence of lymphomas and other cancers have been reported in transplant patients receiving this drug to suppress graft rejection (5). Heretofore these effects have been ascribed to the suppression of the "immunological surveillance" (5). In this report we describe the mutagenic activity of azathioprine for bacteria. In view of the known relationship between mutagenicity and carcinogenic potential (2, 8), it is conceivable that the increased incidence of tumors seen in patients receiving this drug can be ascribed directly to the carcinogenic activity of this agent and not to its effect on immunological surveillance.

MATERIALS AND METHODS

Mutagenicity Assay. The genetic activity of azathioprine was detected using the mutagenicity assay developed by Ames *et al.* (1, 3). Bacteria, *Salmonella typhimurium* TA100 (7) together with the test agents, were incorporated into an agar overlay. Mutants (revertants to histidine independence) were enumerated after incubation at 37° for 48 hr in the dark (9).

Anaerobiosis. To achieve anaerobic conditions, the plates with the tester strains and mutagens were placed in a Gas Pak jar (Baltimore Biological Laboratories, Cockeysville, Md.), the latter was incubated at 37° in the dark for 14 hr, whereupon the plates were removed from the jar and incubated aerobically for an additional 34 hr.

RESULTS

Azathioprine was without significant mutagenic potential (Table 1, Experiment 1) when exposure of the bacteria to the drug was carried out under the standard procedure developed by Ames *et al.* (1, 3). However, when the initial exposure of the bacteria to the test agent was allowed to occur under anaerobic conditions, a mutagenic effect was clearly demonstrable (Table 1, Experiment 1). This effect was dependent on azathioprine concentration (Table 1, Experiment 2).

It was demonstrated that the initial period of anaerobic incubation had no significant effect on the mutagenic activity of a number of agents that display activity under aerobic conditions as well (Table 1, Experiment 3).

DISCUSSION

Recent studies in this laboratory have indicated that the mutagenic potential of nitroheterocyclic substances could be greatly enhanced when the initial incubation was carried out under anaerobic conditions (unpublished results). This observation may reflect the oxygen-labile nature of the postulated hydroxylamino intermediate that is believed to represent an active metabolite (4, 6). Because azathioprine contains a nitro function, and in view of its lack of significant mutagenic activity when tested by the standard (aerobic) procedure, the assay was carried out under anaerobic conditions with a resultant demonstration of mutagenic activity.

Azathioprine is hydrolyzed *in vivo* and, under certain conditions *in vitro*, to 1-methyl-4-nitro-5-thioimidazole, 6-mercaptopurine, and other derivatives (5). It remains to be elucidated whether the mutagenic activity is due to azathioprine *per se* or to one of its metabolites.

The relevance of the present observation to a possible carcinogenic effect in humans remains to be established. The fact that an unusually high proportion of patients receiving azathioprine develop neoplasias suggests a possible relationship.

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Table 1
Mutagenic activity for S. typhimurium TA100

Experiment	Additions	μg/plate	Conditions	Revertants/plate
1	Azathioprine	250	Aerobic	165
	Azathioprine	250	Anaerobic	348
	None	0	Aerobic	109
	None	0	Anaerobic	91
2	None	0	Anaerobic	107
	Azathioprine	25	Anaerobic	114
	Azathioprine	100	Anaerobic	239
	Azathioprine	250	Anaerobic	375
	Azathioprine	400	Anaerobic	553
	Azathioprine	500	Anaerobic	659
	Azathioprine	750	Anaerobic	730
	Azathioprine	1000	Anaerobic	805
	Azathioprine	2500	Anaerobic	33
3	None	0	Aerobic	72
	None	0	Anaerobic	79
	Ethylmethanesulfonate	7	Aerobic	5000
	Ethylmethanesulfonate	7	Anaerobic	5000
	Propyleneimine	1.4	Aerobic	7000
	Propyleneimine	1.4	Anaerobic	7000
	1,2-Epoxybutane	14	Aerobic	430
	1,2-Epoxybutane	14	Anaerobic	450

REFERENCES

1. Ames, B. N. The Detection of Chemical Mutagens with Enteric Bacteria. *In:* A. Hollaender (ed.), Chemical Mutagens, Vol. 1, pp. 267–282. New York: Plenum Press, Inc., 1971.
2. Ames, B. N., Durston, W. E., Yamasaki, E., and Lee, F. D. Carcinogens Are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection. Proc. Natl. Acad. Sci. U. S., *70:* 2281–2285, 1973.
3. Ames, B, N., Lee, F. D., and Durston, W. E. An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens. Proc. Natl. Acad. Sci. U. S., *70,* 782–786, 1973.
4. Cohen, S. M., Alter, A., and Bryan, G. T. Distribution of Radioactivity and Metabolism of Formic Acid 2-[4-(5-Nitro-2-furyl)-2-^{14}C-2-thiazolyl]-hydrazide following Oral Administration to Rats and Mice. Cancer Res., *33:* 2802–2809, 1973.
5. Elion, G. B., and Hitchings, G. H. Azathioprine. Handbuch Exptl. Pharmakol., *48:* 404–425, 1975.
6. McCalla, D. R., Reuver, A., and Kaiser, C. Mode of Action of Nitrofurazone. J. Bacteriol., *104:* 1126–1134, 1970.
7. McCann, J., Spingarn, N. E., Kobori, J., and Ames, B. N. Detection of Carcinogens as Mutagens: Bacterial Tester Strains with R-Factor Plasmids. Proc. Natl. Acad. Sci. U. S., *72:* 979–983, 1975.
8. Miller, E. C., and Miller, J. A. The Mutagenicity of Chemical Carcinogens: Correlations, Problems and Interpretations. *In:* A. Hollaender (ed.), Chemical Mutagens, Vol. 1, pp. 83–119. New York: Plenum Press, Inc., 1971.
9. Speck, W. T., and Rosenkranz, H. S. Base Substitution Mutations Induced in *Salmonella* Strains by Visible Light (450 nm). Photochem. Photobiol., *21:* 369–371, 1975.