Biochemical Pharmacology, Vol. 29, pp. 2533-2536.

0006-2952/80/0915-2533 $02.00/0

EFFECTS OF MISONIDAZOLE ON PURINE METABOLISM IN EHRLICH ASCITES TUMOR CELLS IN VITRO

J. Frank Henderson and George Zombor

Cancer Research Unit and Department of Biochemistry,
University of Alberta,
Edmonton, Alberta, Canada T6G 2H7

(Received 16 May 1980; accepted 30 June 1980)

Misonidazole, or 1-(2-nitro-1-imidazoyl)-3-methoxy-2-propanol, was originally developed as a radiosensitizing agent (1), and is now undergoing clinical trial (2,3). More recently it has been shown to be selectively cytotoxic to hypoxic cells in vitro (4-7) and to have chemotherapeutic effects in vivo (1,8), even in the absence of radiation treatment.

The biochemical basis of misonidazole action is not known. We have considered the possibility that it may act, at least in part, by affecting the energy generating processes and ATP concentrations of cells for the following reasons: (a) it is toxic when cells are dependent on one particular type of energy metabolism (anaerobic glycolysis); (b) like the glycolytic inhibitor, 5-thioglucose (9,10), misonidazole is toxic toward hypoxic cells and this toxicity is potentiated by hyperthermia (11-13); (c) it is activated by reduction of the nitro group (7,14,15) and hence interacts directly or indirectly with cellular oxidation-reduction processes; and (d) some other nitro compounds, such as 2,4-dinitrophenol, are well known inhibitors of energy generating processes. We have therefore studied the effects of misonidazole on ATP generation and catabolism in Ehrlich ascites tumor cells under aerobic and anaerobic conditions, and report here the results of these studies.

Misonidazole was a gift of Dr. D. Chapman, Cross Cancer Institute, Edmonton, Alberta, and was dissolved in 0.154 M sodium chloride for use. Sources of other materials, methods of tumor cell preparation and incubation, and procedures for the separation and measurement of radioactivity in purine bases, ribonucleosides and ribonucleotides have been reported previously (16,17). ATP and its metabolites were separated by thin-layer chromatography, and their radioactivity was measured. The initial total concentration and specific activity of ATP were determined by these methods plus high performance liquid chromatography; it has previously been established (18) that there is no compartmentation of radioactive and non-radioactive ATP with respect to catabolism. Changes in concentrations of metabolites were calculated from the initial specific activity of ATP.

To study ATP catabolism, tumor cells were first incubated with [^{14}C]-adenine to produce radioactive ATP. Unused radioactive adenine was removed by centrifugation and resuspension in fresh medium. Concentrations of radioactive metabolites were measured in cells incubated for an additional 30 min under various conditions, with or without misonidazole (18). Details are given in the legend of Table 1.

The effects of 5 and 10 mM misonidazole on ATP catabolism were studied under aerobic and anaerobic conditions and when cells were depending on three different modes of ATP generation: (a) aerobic glycolysis plus oxidative phosphorylation; (b) oxidative phosphorylation alone; and (c) anaerobic glycolysis alone. Table 1 shows that 5 mM misonidazole did not induce ATP catabolism under any of the conditions used. At 10 mM, however, substantial catabolism was induced on cells incubated aerobically without glucose and in those incubated anaerobically with glucose; however, no catabolism was induced in cells incubated aerobically with glucose.

Table 1. ATP catabolism induced by misonidazole*

Conditions	Misonidazole concn (mM)	ATP concentration (percent of control)
O_2 + glucose	5	106
	10	107
O_2 - glucose	5	97.8
	10	51.7
N_2 + glucose	5	98.5
	10	45.1

*Two ml of a 2% (v/v) Ehrlich ascites tumor cell suspension in calcium-free Krebs-Ringer medium containing 25 mM phosphate with 5.5 mM glucose was incubated in 10 ml Erlenmeyer flasks at 37° with shaking, with an air atmosphere. After 20 min, [8-^{14}C]adenine (ca. 50 mCi/mmole) was added to a final concentration of 100 μM, and incubation was continued for 30 min to synthesize radioactive ATP. Unutilized radioactive adenine was then removed by centrifugation and resuspension of the cells twice in fresh, warmed medium containing glucose. Portions (100 μl total volume) were then incubated with or without misonidazole, under various conditions. Values reported are averages of duplicate measurements and are representative of results obtained in two experiments. Within each experiment, average deviation of individual analyses from the mean was less than 7 percent. Control ATP, 2360 nmoles/g cells. Cells incubated without drug under each condition are used as controls for drug effects under each condition.

In addition to this effect of 10 mM misonidazole on ATP concentrations, both 5 and 10 mM had some effects on certain pathways of purine metabolism. Even in the absence of induced catabolism, there is a certain amount of ATP breakdown during the incubation period. In control cells, the predominant pathway followed is: ATP→ ADP→ adenylate→ inosinate→ inosine→ hypoxanthine. The alternative route to inosine: adenylate→ adenosine→ inosine, normally is a minor process, and because of the presence of adenosine deaminase and adenosine kinase, adenosine does not accumulate to any appreciable degree (19).

Table 2 shows that the conversion of ATP to hypoxanthine + inosine was decreased in cells treated with misonidazole, both when ATP catabolism proceeded at its normal low rate and when it was accelerated by 10 mM misonidazole treatment as described above. Concomitantly, there was an accumulation of adenosine. Such results imply both that the dephosphorylation of adenylate increased relative to its deamination, and that the deamination of adenosine was inhibited; in addition, there would appear to be a defect in the phosphorylation of adenosine. This type of drug effect is quite unusual.

Table 2. Metabolites of ATP in cells treated with misonidazole*

Conditions	Misonidazole concn (mM)	Metabolite concentration (percent of control)		
		Adenine nucleotides	Adenosine	Hypoxanthine + inosine
O_2 + glucose	5	102	287	48.9
	10	106	320	39.2
O_2 - glucose	5	95.7	378	52.3
	10	76.0	316	45.5
N_2 + glucose	5	100	435	48.2
	10	63.4	402	31.3

*Control values (nmoles/g cells): adenine nucleotides, 2736; adenosine, 12; and hypoxanthine + inosine, 87.

To confirm and expand these observations, further experiments were done using cells incubated with a mixture of glucose plus 2-deoxyglucose which would, itself, induce a moderate degree of ATP catabolism (18). This situation would both stress the cells with respect to ATP generation, increase the flow of metabolites along catabolic pathways, and also produce adenosine endogenously. The latter factors would make it easier to study the effects of a range of concentrations of misonidazole on the catabolic pathways.

Table 3 shows that, in the absence of misonidazole, ATP catabolism was induced and total adenine nucleotides were decreased; adenosine concentrations increased only very slightly, and the major end products of ATP catabolism were hypoxanthine + inosine. In the presence of 1, 5 or 10 mM misonidazole, there was an additional, dose-related induction of ATP and total adenine nucleotide catabolism. As in the previous experiments, there was an increased accumulation of adenosine, which must be due at least to an inhibition of adenosine deaminase in misonidazole-treated cells; in addition, adenosine phosphorylation may be decreased. Under the conditions of these experiments there was no decrease in the formation of hypoxanthine + inosine, but of course the flow along the catabolic pathways was considerably greater than in the experiments of Tables 1 and 2. This result indicates that the effect of misonidazole on the relative rates of deamination and dephosphorylation of adenylate is small or perhaps even non-existent under these conditions.

Table 3. Effects of misonidazole on ATP catabolism in cells incubated with glucose + deoxyglucose*

Misonidazole concn (mM)	Metabolite concentrations (percent of control)							
	ATP		Adenine nucleotides		Adenosine		Hypoxanthine + inosine	
	O_2	N_2	O_2	N_2	O_2	N_2	O_2	N_2
0	54.8	33.2	57.7	36.5	117	106.7	263	342
1	32.4	30.2	38.4	35.4	238	137	379	455
5	24.8	27.2	30.1	31.6	296	218	427	369
10	19.1	22.4	25.4	29.9	474	298	600	407

*Cells were incubated with 0.69 mM glucose + 4.81 mM deoxyglucose.

These studies have shown that short exposures of Ehrlich ascites tumor cells to moderate concentrations (1-5 mM) of misonidazole did not lead to ATP catabolism, whether by direct effects or indirectly by interfering with one or another energy generating process. Higher concentrations, however, did induce ATP catabolism when the cells were depending either on oxidative phosphorylation or anaerobic glycolysis alone, but not when cells were using both aerobic glycolysis plus oxidative phosphorylation. Here it may be noted that different cell types have been reported (7) to vary considerably in their sensitivity to the effects of misonidazole. Cytotoxicity experiments commonly expose cells to misonidazole for much longer than the period used here, and the possibility should be considered that lower concentrations might have detrimental effects upon prolonged exposure. Finally, the experiments using deoxyglucose suggest that cells in which rates of energy generation processes are partially impaired or are naturally lower than in the tumor cells used might have an increased sensitivity to induction of ATP catabolism by misonidazole.

Even at relatively low concentrations, misonidazole affected the pathways of adenylate and adenosine metabolism, leading to accumulation of adenosine and a decrease in the formation of hypoxanthine and inosine. The basis of this effect includes at least partial inhibition of adenosine deaminase, and presumably one or another enzyme of adenylate metabolism as well. Studies of isolated enzymes are called for, but for these the possibility that the inhibitory effects might be caused by metabolites of misonidazole, rather than by the parent drug, needs to be considered; such metabolites were not available for study.

The relationship of the effects observed here to either the cytotoxic effects of misonidazole or to its host toxicity remains to be evaluated. The fact that misonidazole was somewhat more potent under anaerobic conditions than in air would suggest that these effects may be pharmacologically important. Similarly, host toxicity related to lowered ATP concentrations, the accumulation of adenosine, or both, may be envisioned.

Acknowledgement---This work was supported by the National Cancer Institute of Canada.

REFERENCES

1. J.F. Fowler, G.E. Adams and J. Denekamp, Cancer Treat. Rev. 3, 227 (1976).

2. A.J. Gray, S. Dische, G.E. Adams, I.R. Flockhart and J.L. Foster, Clin. Radiol. 27, 151 (1976).

3. R.Y. Tomlinson, S. Dische, A.J. Gray and I.M. Errington, Clin. Radiol. 27, 167 (1976).

4. E.J. Hall and L. Roizin-Towle, Radiology 117, 453 (1975).

5. B.A. Moore, B. Palcic and L.D. Skarsgard, Radiat. Res. 67, 459 (1976).

6. C.R. Geard, S.F. Povlas, M.B. Astor and E.J. Hall, Cancer Res. 38, 644 (1978).

7. Y.C. Taylor and A.M. Rauth, Cancer Res. 38, 2745 (1978).

8. J. Denekamp and H.R. Harris, Radiat. Res. 61, 191 (1975).

9. S.H. Kim, J.H. Kim and E.W. Hahn, Cancer Res. 38, 2935 (1978).

10. C.W. Song, J.J. Clement and S.H. Levitt, Cancer Res. 38, 4499 (1978).

11. E.J. Hall, M. Astor, C. Geard and J. Biaglow, Br. J. Cancer 35, 809 (1977).

12. E.J. Hall and J. Biaglow, Int. J. Radiat. Oncol. Biol. Phys. 2, 521 (1977).

13. I.J. Stratford and G.E. Adams, Br. J. Cancer 35, 807 (1977).

14. G.E. Adams, I.R. Flockhart, C.E. Smithers, I.J. Stratford, P. Wardman and M.E. Watts, Radiat. Res. 67, 9 (1976).

15. A.J. Varghese, S. Gulyas and J.K. Mohindra, Cancer Res. 36, 3761 (1976).

16. G.W. Crabtree and J.F. Henderson, Cancer Res. 31, 985 (1971).

17. F.F. Snyder and J.F. Henderson, J. Cell. Physiol. 82, 349 (1974).

18. C.A. Lomax, A.S. Bagnara and J.F. Henderson, Can. J. Biochem. 53, 231 (1975).

19. C.A. Lomax and J.F. Henderson, Cancer Res. 33, 2825 (1973).