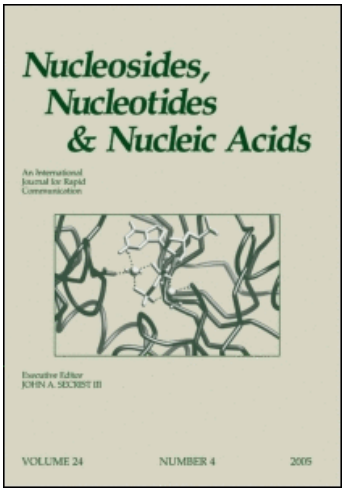

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Increased Nucleoside Concentrations in Tumors as Markers of Tiazofurin Action

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INCREASED NUCLEOSIDE CONCENTRATIONS IN TUMORS AS MARKERS OF TIAZOFURIN ACTION

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Abstract. Tiazofurin injection (150 mg/kg, i.p.) into rats bearing hepatoma 3924A increased in the tumor the pools of guanine, uridine, thymine, hypoxanthine, inosine, and thymidine 3.5-, 4.4-, 8.0- 18.4-, 18.7- and 42-fold over the controls. There were only minor changes in the host liver. This is the first report showing a selective action of tiazofurin in cancer cells on the concentrations of nucleosides and bases, indicating that these might be used as markers of the impact of tiazofurin in clinical trials.

Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC 286193) (TR) exhibits potent antitumor activity against murine tumors including leukemias and the Lewis lung carcinoma (1). In tumors sensitive to tiazofurin, the drug is metabolized to an analog of NAD, TAD (thiazole-4-carboxamide adenine dinucleotide), which strongly inhibits IMP dehydrogenase and subsequently depletes guanylate pools, resulting in the inhibition of tumor cell growth and proliferation (2-5). Tiazofurin exhibited marked cytotoxic and antitumor activity against subcutaneously implanted hepatoma 3924A in rats (6). Tiazofurin injection into rats bearing hepatoma 3924A resulted in a significant decrease in the tumors of guanylate pools (GMP, GDP, GTP and dGTP) with a simultaneous elevation of IMP content (6). Since tiazofurin inhibited *de novo* synthesis of guanylates, the salvage of precursors of nucleotides, namely, nucleobases and nucleosides, might play a significant role in determining the sensitivity of tumors to the drug. To elucidate the relative importance of the precursors of nucleotides, the *in vivo* concentrations of some of the biologically relevant nucleobases and nucleosides were determined in hepatoma 3924A and in liver. The results presented here should aid in monitoring the responsiveness or resistance to tiazofurin of tumors in clinical trials.

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MATERIALS AND METHODS

Materials: Tiazofurin was provided by Dr. V. Narayanan, National Cancer Institute, Bethesda, MD. Base and nucleoside standards were obtained from Sigma Chemical Co., St. Louis, MO. Radial-Pak Resolve C_{18}, 5 μ and Partisil 10-SAX columns (8 mm x 10 cm) were purchased from Waters Associates, Milford, MA.

In Vivo Studies: Rapidly growing solid hepatoma 3924A was maintained as bilateral subcutaneous transplants in male ACI/N rats (180-200 g). The biological and biochemical properties of the hepatomas have been reported (7). Rats bearing 2-week old tumors were injected intraperitoneally with a dose of tiazofurin (150 mg/kg); 6 hr later the animals were lightly anesthetized with ether, and the hepatoma and liver were freeze-clamped as previously described (8). Livers from normal male ACI/N rats (180-200 g) were also freeze-clamped using the same procedure.

Analysis of IMP and GTP: After freeze-clamping, tissues were extracted with cold 10% trichloroacetic acid (TCA) (1:5 w/v), immediately neutralized with 0.5 M tri-n-octylamine in freon (9). An aliquot of the neutralized extract was analyzed on a Partisil 10-SAX column of Waters HPLC unit, using the ammonium phosphate buffer system (10). Under the conditions of analysis, IMP eluted at 12 min and GTP at 39 min.

Nucleobase and Nucleoside Analysis: Freeze-clamped tissues were powdered under liquid nitrogen and then were extracted with cold 10% TCA (1:5 w/v) for 10 min. The extract, after centrifugation at 12,000 x g for 3 min, was neutralized immediately with tri-n-octylamine. An aliquot of the extract was analyzed on Waters HPLC system by loading on a Partisil 10-SAX column, preequilibrated with 1 mM ammonium acetate, pH 4.7, to separate nucleobases and nucleosides from nucleotides using an isocratic elution for 10 min with the same buffer at a flow rate of 2 ml/min. Nucleobases and nucleosides elute in the void volume, whereas nucleotides are charged and adhere to the column. The fractions containing the nucleobases and nucleosides were collected, pooled, frozen, and immediately lyophilized.

The lyophilized fraction was dissolved in water, neutralized with tri-n-octylamine and an aliquot of the sample (0.2 ml) was analyzed for the nucleobase and nucleoside content, as described elsewhere (11). In short, the samples were loaded on a Radial-Pak C_{18} column of Waters HPLC unit preequilibrated with 1 mM sodium acetate, pH 3.9 (buffer A) and eluted isocratically with the same buffer for 4 min at a flow rate of 1 ml/min and then 0-6% linear gradient was applied for 16 min with acetonitrile. The buffer system was then switched to 5 mM sodium acetate, pH 5.4 (buffer B) over a period of 1 min. A linear gradient (0-10%) with buffer B and acetonitrile was applied for 19 min and then maintained at 10% concentration for 5 min. The column was regenerated with a linear gradient containing buffer A and 60% acetonitrile for 5 min at a flow rate of 2 ml/min and then equilibrated with buffer A for 5 min before analyzing the next sample. Absorption of the compounds was monitored at 254 and 280 nm. Under the conditions of the analysis the following compounds eluted (min): uric acid (11.4); hypoxanthine (14.8); uridine (15.5); xanthine (16.6); thymine (18.3); deoxyuridine (19.9); guanine (20.8); inosine (22.5); xanthosine (25.7); thymidine (26.7); deoxycytidine (34.2); adenine (38.6); adenosine (41.9); and deoxyadenosine (44.3). The identity of the compounds was verified by retention time, peak height ratio and overlay with known amount of standards as described elsewhere (11).

RESULTS AND DISCUSSION

Concentrations of nucleobases and nucleosides were examined in the livers of normal and hepatoma 3924A bearing rats (host liver). Since there was no significant difference between the pools of nucleosides and nucleobases in

normal and host livers (Table 1), host liver was used for further comparison with hepatoma 3924A.

The effect of tiazofurin injection on IMP and GTP concentrations in hepatoma 3924A and host liver is presented in Table 2. The results demonstrate an 18-fold increase in the IMP pools with concurrent depression of GTP concentration to 25% in the hepatoma. Tiazofurin produced only minor effects on the host liver, increasing IMP pools 3.7-fold without significantly reducing GTP pools. The ratio of the concentration of IMP to GTP, an indicator of tiazofurin action, increased 77-fold in hepatoma following tiazofurin injection, compared to 6-fold in the host liver.

In hepatoma 3924A cells, incubation with tiazofurin decreased the flux of radiolabelled formate into IMP and the salvage of guanine to guanylates; the latter effect was attributed to an inhibition of the purine salvage by the

Table 1: Concentrations of nucleobases and nucleosides in livers of normal and hepatoma bearing rats[a]

Nucleobase or nucleoside	Normal liver nmol/g	Host liver nmol/g	% of normal liver
Adenine	22.0 ± 0.9	20.2 ± 1.9	92
Adenosine	4.6 ± 1.0	6.8 ± 2.1	148
Guanine	1.1 ± 0.3	1.0 ± 0.2	91
Inosine	1.5 ± 0.2	1.3 ± 0.2	87
Hypoxanthine	1.0 ± 0.2	1.3 ± 0.1	130
Xanthine	1.8 ± 0.3	1.6 ± 0.3	89
Deoxycytidine	39.2 ± 3.2	35.5 ± 7.3	90
Thymine	0.1 ± 0.05	0.1 ± 0.05	100
Thymidine	1.1 ± 0.3	1.2 ± 0.3	109
Uridine	2.6 ± 0.8	1.8 ± 0.7	69

[a]Normal or hepatoma 3924A bearing rats were lightly anesthetized; the livers were freeze-clamped and the samples were analyzed as detailed in the Materials and Methods. Values are expressed as means ± S.E. of 4 or more animals.

high concentrations of IMP that accumulated in the cells following tiazofurin treatment (12,13). This idea is supported by evidence that IMP in micromolar concentrations inhibited in hepatoma extracts, in a dose-dependent fashion, the activities of hypoxanthine- and guanine phosphoribosyltransferase without any effect on the activity of adenine phosphoribosyltransferase (6). From these enzymic investigations it was postulated that when IMP concentrations are high after tiazofurin injection and in the hepatoma the salvage enzymes are inhibited *in vivo*, then guanine and hypoxanthine should accumulate in the tumor. We tested this hypothesis and it was found that six hr after tiazofurin injection the concentrations of guanine and hypoxanthine increased 3.5- and 18.7-fold, respectively, over the control values (Table 3). Thus the freeze-clamp studies, through *in vivo* determination of nucleoside concentrations, supported the indications obtained in the *in vitro* enzyme extract studies.

The striking elevation in inosine concentration (18.7-fold) might have resulted from the degradation of the expanded pools of IMP by nucleotidase and/or phosphatase activity. The fact that the concentrations of adenine and adenosine did not change significantly after tiazofurin injection agrees with

Table 2: Effect of tiazofurin treatment on IMP and GTP concentrations in hepatoma 3924A and host liver, *in vivo*[a]

Nucleotides	Host liver		% of	Hepatoma 3924A		% of
	control	TR treated	control	control	TR treated	control
IMP	68.6 ± 31.4	250.7 ± 59.8	365[b]	14.5 ± 1.9	262.0 ± 30.1	1807[b]
GTP	269.4 ± 25.5	187.8 ± 17.7	70	340.5 ± 37.4	85.2 ± 3.1	25[b]
IMP/GTP ratio	0.25	1.46	584	0.04	3.07	7675

[a] Male ACI/N rats bearing hepatoma 3924A were injected intraperitoneally with 150 mg/kg tiazofurin; 6 hr later animals were lightly anesthetized, tumors and liver were excised and freeze-clamped. The freeze-clamped tissue was ground to a powder which was extracted and analyzed on HPLC as detailed in the Materials and Methods. Values are expressed as nmol/g ± S. E. of 4 or more animals.

[b] Significantly different from controls ($p < 0.05$).

Table 3: Effect of tiazofurin treatment on the content of nucleobases and nucleosides in the host liver and in hepatoma 3924A, *in vivo*[a]

Nucleobase or Nucleoside	Host liver			Hepatoma 3924A			
	control nmol/g	tiazofurin nmol/g	% of control	control nmol/g	% of host liver	tiazofurin nmol/g	% of control
Adenine	20.2 ± 1.9	15.0 ± 2.3	74	12.0 ± 1.8	59[b]	10.6 ± 1.6	88
Adenosine	6.8 ± 2.1	4.0 ± 2.5	59	0.5 ± 0.1	7[b]	0.5 ± 0.05	100
Guanine	1.0 ± 0.2	1.2 ± 0.2	120	1.5 ± 0.2	150	5.2 ± 0.7	347[b]
Inosine	1.3 ± 0.2	3.3 ± 0.6	254	1.9 ± 0.2	146	35.6 ± 7.6	1874[b]
Hypoxanthine	1.3 ± 0.1	1.1 ± 0.2	85	2.5 ± 0.4	192[b]	45.9 ± 4.6	1836[b]
Xanthine	1.6 ± 0.3	1.8 ± 0.4	112	4.7 ± 0.8	294[b]	10.9 ± 0.9	232[b]
Deoxycytidine	35.5 ± 7.3	18.6 ± 2.9	52	31.9 ± 7.8	90	33.6 ± 10.1	105
Thymine	0.1 ± 0.05	0.2 ± 0.1	200	0.3 ± 0.05	300[b]	2.4 ± 0.4	800[b]
Thymidine	1.2 ± 0.3	1.6 ± 0.4	133	0.2 ± 0.05	17[b]	8.4 ± 0.9	4200[b]
Uridine	1.8 ± 0.7	2.1 ± 0.5	117	1.4 ± 0.2	78	6.2 ± 0.9	443[b]

[a] Techniques used were the same as in the legend to Table 2. Values are expressed as means ± S.E. of 3 or more animals.

[b] Significantly different from controls ($p < 0.05$).

the earlier observation that the adenylate pools did not alter after tiazofurin treatment (6). The marked elevations of thymine and thymidine (8-and 42-fold) following tiazofurin injection might reflect the catabolism of the dTTP pools which could not be utilized for DNA biosynthesis because of the marked depletion of the dGTP content produced by this drug (14). The accumulation of thymidine and thymine may also be attributed to the very low activity in this hepatoma of dihydrothymine dehydrogenase (EC 1.3.1.2), the rate-limiting enzyme of thymidine degradation (7). The 4-fold increased uridine concentration may relate to the tiazofurin-induced elevation in UTP pools in the hepatoma, reflecting a possible degradation of this nucleotide.

These studies demonstrate that tiazofurin treatment in the rat caused striking increases in the hepatoma of the concentrations of certain nucleic acid precursors. The novel observations indicate that the pools of guanine, uridine, thymine, hypoxanthine, inosine, and thymidine were elevated 3.5-, 4.4-, 8.0-, 18.4-, 18.7-, and 42-fold over the controls. Thus, these nucleosides and nucleobases may be used as markers of tiazofurin action and could be useful in monitoring the sensitivity or resistance to tiazofurin in clinical trials.

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REFERENCES

1. Robins, R. K., Srivastava, P. C., Narayanan, V. L., Plowman, J. and Paull, K. D. (1982) J. Med. Chem. 25, 107-108.
2. Jayaram, H. N., Dion, R. L., Glazer, R. I., Johns, D. G., Robins, R. K., Srivastava, P. C. and Cooney, D. A. (1982) Biochem. Pharmac. 31, 2371-2380.
3. Jayaram, H. N., Smith, A. L., Glazer, R. I., Johns, D. G. and Cooney, D. A. (1982) Biochem. Pharmac. 31, 3839-3945.
4. Cooney, D. A., Jayaram H. N., Gebeyehu, G., Betts, C. R., Kelley, J. A., Marquez, V. E. and Johns, D. G. (1982) Biochem. Pharmac. 31, 2133-2136.
5. Cooney, D. A., Jayaram, H. N., Glazer, R. I., Kelley, J. A., Marquez, V. E., Gebeyehu, G., Van Cott, A. C., Zwelling, L. A. and Johns, D. G. (1983) Advan. Enzyme Regul. 19, 87-102.
6. Lui, M. S., Faderan, M. A., Liepnieks, J. J., Natsumeda, Y., Olah, E., Jayaram, H. N., and Weber, G. (1984) J. Biol. Chem. 259, 5078-5082.
7. Weber, G. (1983) Cancer Res. 43, 3466-3492.
8. Weber, G., Stubbs, M. and Morris, H. P. (1971) Cancer Res. 31, 2177-2183.
9. Khym, J. X. (1975) Clin. Chem. 21, 1245-1252.

10. Liepnieks, J. J., Faderan, M. A., Lui, M. S. and Weber G. (1984) Biochem. Biophys. Res. Commun. 122, 345-349.
11. Pillwein, K., Jayaram, H. N. and Weber, G. (1986) Cancer Res. submitted for publication.
12. Weber, G., Lui, M. S., Jayaram, H. N., Pillwein, K., Natsumeda, Y., Faderan, M. A. and Reardon, M. A. (1985) Advan. Enzyme Regul. 23, 81-102.
13. Jayaram, H. N., Pillwein, K., Lui, M. S., Faderan, M. A. and Weber, G. (1986) Biochem. Pharmac. 35, 587-593.
14. Weber, G., Natsumeda, Y., Lui, M. S., Faderan, M. A., Liepnieks, J. J. and Elliott, W. L. (1984) Advan. Enzyme Regul. 21, 53-69.

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